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THE PROBLEM OF REJUVENESCENCE IN PROTOZOA*

LORANDE LOSS WOODRUFF

It is a pleasure to accept the invitation to present a summary of the results derived from our genetic cultures of *Paramœcium* at Yale, with special reference to the bearing of this and other work on the problem of rejuvenescence in protozoa; for, it seems to me, the problem has now passed successfully through the periods of youth and adolescence, and is approaching that of maturity, when we may confidently expect the production of some conclusions of general significance.

Although the problems of protoplasmic senescence and the function of conjugation have afforded the stimulus for investigations on the life history of infusoria since Ehrenberg, nearly a century ago, theorized on the potential 'immortality' of those forms, we may take, as the point of departure for our present brief review of the subject, the classical experimental studies of Maupas. As is well known Maupas' studies afforded a wealth of data, all of which indicated that continued reproduction by division results in degeneration and death, and seemed to place the conclusion, that conjugation is a *sine qua non* for the life of infusoria, upon a firm empirical basis.

A series of important investigations by Hertwig and Calkins confirmed Maupas' general conclusion that infusoria, after a more or less definite number of divisions, degenerate and finally die if conjugation is prevented. Calkins, however, made the significant discovery that artificial stimuli of different kinds may, for a time, be substituted with success for conjugation since, by the opportune use of artificial stimulation, he was able to prolong the life of one culture of *Paramœcium caudatum* to the 742d generation.

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* Presented at the symposium on protozoology, Amer. Ass'n. Adv. Science, Berkeley, Cal., August 5, 1915. For a review of the earlier work on this subject, at Yale, see BIOCHEMICAL BULLETIN, 1912, i, p. 396.

Enriques studied the same general problem and reached the conclusion that the degeneration and death of infusorian cultures was due to bacterial poisons, because he succeeded in breeding *Glaucoma scintillans* for 683 generations without signs of degeneration when he took measures to eliminate this factor. Whether this was the crucial factor in his method is open to question, but the significant fact remains that his animals survived nearly twice as long as those of earlier workers, without conjugation or artificial stimulation, thus suggesting that, if suitable conditions are supplied, reproduction by division can proceed indefinitely.

At this point I took up the problem and first investigated the possibility that the degeneration observed in the previous investigations was induced by too great uniformity in the conditions of culture, or by the culture-medium being deficient in something essential for the continued well-being of the organisms. A race of *Paramœcium aurelia* was isolated in 1907 and bred on infusions of various materials found in the natural environment of the animal, while a sub-culture was subjected to the relatively constant hay-infusion culture-conditions generally employed. The result was that the cells bred in the constant hay-infusion medium died out after a typical Calkins cycle, while those bred on the 'varied-environment' medium did not pass through periods of marked physiological depression or show morphological changes which could be interpreted as abnormal. This race is still, after more than eight years in culture, in a normal condition, having attained over 5250 generations without conjugation or the use of artificial stimuli.

The success with the varied culture medium naturally led to the question whether the longevity of *Paramœcium*, on a varied environment, is dependent upon intrinsic stimuli from the frequent changes of the medium, or whether a constant medium of hay-infusion is unfavorable because it lacks some elements which are essential for the continued existence of the organism. Accordingly,[1] a sub-culture of this race was bred for a period of nine months on a constant culture-medium of beef-extract. The continued health of the organisms on this constant medium throughout the experiment, which was continued sufficiently long to include a Calkins cycle, if such was inherent, indicated that it is the composition of the medium, rather than the changes in the medium, which is conducive to the unlimited development of this race without the necessity of conjugation or artificial stimulation.

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[1] Woodruff and Baitsell: *Journ. of Exp. Zool.*, 1911.

From a study of various species of hypotrichous infusoria, as well as the main culture of *Paramœcium aurelia,* it was found that minor periodic rises and falls of the division-rate occur, from which recovery is autonomous. These fluctuations were termed 'rhythms' and contrasted with the so-called cycle, which comprises a varying number of rhythms and, according to Maupas and Calkins, ends in the death of the race, if conjugation or artificial stimulation is not resorted to.

The problem of rhythms was then studied intensively.[2] It was found that the subjection of the culture to the most constant environmental conditions failed to eliminate the rhythms and thus to resolve the graph of the multiplication rate into an approximately straight line; but, instead, the rhythms appeared slightly more pronounced. It was also found, from a study of the temperature coefficient[3] of the rate of reproduction of the culture, that this is influenced by temperature at a velocity similar to that for a chemical reaction, except when the rhythms interfere. Thus, it is apparent that there are inherent rhythmical changes in the phenomena of the cell which produce slight fluctuations in the division-rate.

The results, then, from the study of this pedigreed race of *Paramœcium aurelia* led us to conclude that this organism, when subjected to suitable culture conditions, has the power of unlimited reproduction by division without conjugation or artificial stimulation; the only necessary variation in the rate of reproduction being the normal minor periodic rise and fall of the division-rate, due to some unknown factor in cell phenomena, from which recovery is autonomous (rhythm).

Calkins,[4] however, did not share this optimism and sought the explanation, of the diametrically opposite results derived from his and from our cultures of *Paramœcium,* in variations in the tendency to conjugate, which he and Jennings had found to exist in different races of this organism. Thus, Calkins emphasized the fact that he could readily induce conjugation in his culture, whereas experiments to secure conjugation in our cultures were without effect. He, therefore, stated that "the two races cannot be compared in regard to vitality, since normal conjugation was prevented in the conjugating race, whereas in the non-conjugating race there has been no artificial prevention of a normal process."

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[2] Woodruff and Baitsell: *Journ. of Exp. Zool.,* 1911.

[3] Woodruff and Baitsell: *Am. Journ. Physiology,* 1911.

[4] Calkins: *Journ. of Exp. Zool.,* 1914.

With this issue raised, it was essential to determine whether our race was actually non-conjugating. Accordingly, a more extensive series of mass cultures were started from it, with the result that conjugants were finally secured, thus demonstrating that this race is a conjugating race when the proper conditions for conjugation are realized. Therefore, there is no evidence extant that a non-conjugating race of *Paramœcium* exists.

In a recent paper, Calkins[5] states that possibly his terms "conjugating" and "non-conjugating" were not happily chosen, and that he merely meant to indicate that some races are more prone to conjugate than others. Admitting this interpretation of his terms, they express a fact. But this interpretation begs the question which his suggestion was advanced to explain.

With this theory eliminated, the results derived from this culture demonstrate, we believe, that the very limited periods in which Maupas, Calkins, and others observed degeneration, have no significance for the question as to whether degeneration and death are inevitable results of reproduction without conjugation. In other words, this one positive result from this race outweighs all the negative evidence derived from work on the infusoria, and justifies the statement that these organisms can live indefinitely, when subjected to favorable environmental conditions, without conjugation or artificial stimulation.

With conjugation eliminated as a necessary factor in the life history, obviously the next point to be elucidated, if possible, was the underlying factor inherent in the cell, the physiological expression of which is the rhythm. Although morphological or physiological variations that could be interpreted as the result of degeneration were never observed in this race of *Paramæcium,* we early noted "that various nuclear changes which are not at present recognized occur normally in the life history of *Paramæcium*"; and we suggested that possibly, when conjugation is prevented, a reorganization of the nuclear apparatus within the individual cell occurs.[6] Erdmann independently reached an essentially similar position from a consideration of the published data on this culture and a critical study of infusorian life histories; and further, in an experimental study of *Amœba diploidea,* suggested that a relation exists between sexual phenomena and rhythms.[7] Accordingly, we collaborated in a study of the *daily* cytological changes of this race of *Paramæcium* during a period of six months, and discovered that the rhythms in the division-rate are the physiological expression of internal phenomena which involve the formation of a complete new nuclear apparatus, by a definite sequence of normal morphological changes that simulate conjugation.[8] This nuclear reorganization, which we term *endomixis,* consists, in essence, of a gradual disintegration and absorption of the macronucleus in the cytoplasm. Simultaneously, a multiplication of the micronuclei is in progress. Certain of the resulting micronuclei degenerate while the remaining one (or two) form the new macronuclear and micronuclear apparatus. This results in the reorganization of the cell without the fusion of two animals.

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[5] Calkins: *Am. Naturalist*, 1915.

An essential morphological difference between endomixis and conjugation is the absence of the third micronuclear division, which, in conjugation, forms the stationary and migratory micronuclei; and, of necessity, the non-formation of a *syncaryon.* After conjugation the reorganized cell has a new macronuclear and micronuclear apparatus, composed of combined material from the conjugants, while, after endomixis, the reorganized cell has a new macronuclear and micronuclear apparatus composed of material from its own micronuclei. In a word, the essential distinctive features of endomixis are the absence of the third micronuclear division and the absence of the introduction of foreign nuclear (and cytoplasmic) material into the cell.

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[6] Woodruff: *Amer. Naturalist,* 1908.

[7] Erdmann: *Ergeb. d. Anat. u. Ent.,* 1908; *Archiv. f. Protistenk.,* 1913.

[8] Woodruff and Erdmann: *Proc. Soc. Exp. Biol. and Med.,* 1914; *Journ. Exper. Zool.,* 1914; also Erdmann and Woodruff: *Biol. Cent.,* 1914.

For reasons advanced elsewhere, we hold that endomixis is not parthenogenesis, but it is not necessary at this time to enter into a more or less academic discussion in regard to the exact classification of endomixis among *Entwicklungserregung* phenomena.

In the light of the discovery of the details of endomixis, by the daily study of pedigreed cells of this race, a survey of the cells, which had been preserved at intervals during the previous seven years of its life, revealed a number of the crucial stages of endomixis, thus showing that the process has been in progress ever since the race has been bred, and is not, as Hertwig[9] suggests, a development during long subjection to culture. That it is not even a peculiarity of this race is evident from the fact that we have found endomixis in four other distinct races selected at random—three from America and one from Germany. Further, Hertwig, in 1889, incidentally noted in a mass culture, in which conjugation had not been observed for a long time, certain animals whose nuclear structure apparently indicated isolated stages of the process which have been elucidated in our cultures. Therefore, it seems well established that endomixis is of general and probably universal occurrence in *Paramœcium aurelia.* It also occurs, with essentially similar features, in all the races of *Paramœcium caudatum* which we have studied.[10]

Now, in regard to the significance of endomixis from the standpoint of our subject—rejuvenescence in protozoa: It is clear that the cycle emphasized by Maupas, Calkins and others, is merely a phantom which has continually receded as each successive investigator has approached the problem with improved culture methods, until it has vanished with this eight-year-old culture. What remains then is the rhythm and in the light of endomixis—the underlying cytological phenomenon of which the rhythm is an outward physiological expression—the whole problem takes on a new aspect. The cell automatically reorganizes itself periodically by a process which, in its main features, simulates conjugation, but without a contribution of nuclear material from another cell.

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[9] Hertwig: *Biol. Cent.,* 1914.

[10] Erdmann and Woodruff: *Journ. Exp. Zool.,* 1916.

At the present stage of our knowledge, the rhythm may perhaps be considered as an expression of a sort of temporary "senescence," for which is provided an internal, automatically working, antidote in the form of endomixis. But, if one considers this as a "senescence"-phenomenon and endomixis a "rejuvenation"-phenomenon, then it is equally permissible so to consider the momentary fluctuating periods of anabolic and catabolic ascendency in the metabolism of the cell. But I would point out that it is a case of trying to force new wine into old bottles in order to save an idea, and that this line of reasoning, pushed a little farther, approaches perilously near a *reductio ad absurdum*. One certainly must grant that the prevalent idea of "senescence" in infusoria is far removed from this subtle, automatically eliminated type which rhythms may indicate.

The work at Yale in the past has been to determine whether *conjugation* is a necessary factor in the life of infusoria; and we believe that 5250 generations without conjugation is *strong* evidence in the negative. But, obviously, because conjugation is not necessary in the life of *Paramœcium* under favorable environmental conditions, it does not follow that conjugation is not necessary under other conditions, or that it does not have a "rejuvenating" function. There is nothing mutually exclusive in the *fact* that conjugation is not necessary and the *idea* that conjugation has a dynamic function when it occurs. In fact we have *always* leaned toward and definitely stated the view that conjugation probably has a dynamic function, which is important when the organisms are subjected to unfavorable conditions. Calkins has secured some evidence which indicates that, after conjugation, all the processes of the cell including reproduction proceed with greater vigor; and thus he substantiates the view of Bütschli, Maupas, Hertwig, and others. Jennings, on the other hand, definitely states that there is no evidence from his work that conjugation in the infusoria increases the reproductive power of, or rejuvenates, the organism physiologically in any way and puts all the emphasis on the side of variation and heredity.

However, since the cytological phenomena of conjugation, with the exception of syncaryon formation, are so similar in their broad features with those of endomixis, and since accelerated vital activities including reproduction do *follow* endomixis, it seems reasonable to believe that accelerated vital phenomena follow conjugation—that is, that both processes, broadly speaking, "rejuvenate" the organism physiologically. Both processes afford opportunity for a rearrangement of the molecular constitution of the cell, conjugation affording amphimixis and endomixis affording *endo*mixis.

To recent contentions that our conclusions were wrong, in regard to conjugation not being a necessity for the continued reproduction of infusoria, we would reply that endomixis is *not* conjugation; and no one had any other phenomenon than conjugation, involving syncaryon formation, in mind until the discovery of endomixis, in which a syncaryon is not formed. To say that endomixis fills essentially the same rôle as conjugation in the infusorian life-history is to beg the entire question. In a word, the whole aspect of the problem of senescence and rejuvenescence in protozoa has changed with our knowledge of endomixis. The question is now not whether conjugation is necessary—for it is not—but whether endomixis is necessary. If endomixis is necessary, as it may well be, and *if* one feels justified in considering the physiological phenomena which are synchronous with the start of endomixis as evidence of "senescence" and those synchronous with the end of endomixis as indicating "rejuvenation"—then, this is a radically *new* phase of the old idea of protoplasmic senescence and rejuvenescence in the infusoria.

Osborn Zoological Laboratory,
Yale University.

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January, 1916

3. *The origin and structure of a fibrous tissue formed in wound healing.* (Illustrated and with demonstrations.) George A. Baitsell, introduced by R. G. Harrison, Yale University.

In a previous paper (Jour. Exp. Med., vol. 21, pp. 455–479) the author was able to show that in living cultures of adult frog tissues there occurs, in many instances, a transformation of the plasma clot in which the living tissue is imbedded. This transformation results in a consolidation or fusion of the elements of the fibrin net and a consequent formation from it of a fibrous tissue which is identical in its form and structure and in many of its staining reactions with a regular collaginous connective tissue. It appeared evident that, whether or not the fibrous tissue directly formed from the fibrin clot remained as a permanent connective tissue, such a reaction must play an important part in wound healing. The present paper gives the results obtained from an extensive series of experiments undertaken with the purpose of studying the action and fate of the fibrin clot formed during wound healing and they may be briefly summarized as follows:

1. In experimental wounds in frog skin made by removing various sized pieces of skin from the animal there is a rapid coagulation of the blood plasma and lymph to form a coagulation tissue which fills the wound cavity.

2. The observations on living animals show that if the animal remains quiet for a few minutes after an operation the coagulation tissue becomes resistant and is of sufficient strength to hold the cut edges of the wound in place and to retain its position in the wound cavity. The coagulation tissue is also resistant to the action of water and the animal can be placed in an aquarium a few minutes after an operation has been performed without any injury to the coagulation tissue which serves, at least temporarily, as a connective tissue and as a base for the epithelial cells which rapidly move in from all the cut edges to close the wound cavity. Observations have been made on the healing of various types of wounds viz., 1) wounds in which only a cut was made in the skin and no tissue was removed, 2) wounds in which pieces of skin of various sizes were removed and 3) wounds in which pieces of skin were removed and either another piece of skin or the stratum compactum obtained by pancreatin digestion of a piece of adult frog skin were implanted. In all these types of wounds the coagulation tissue formed by the clotting of the lymph or blood plasma was an essential feature of the rapid healing which occurred since it filled the wound cavity and held all the cut edges in their proper positions.

3. The study of prepared sections show that, at first, in the coagulation tissue, formed in the wound as a result of the clotting of the blood and lymph, a typical fibrin net is present. Later this fibrin net is transformed into fibrous tissue containing bundles of wavy fibers in which, in many instances, the individual fibrils can be noted. In the course of a few days the entire clot becomes transformed into this new fibrous tissue. This transformation of the clot and the formation of the fibrous tissue is not due to any intracellular action inasmuch as it take place before the connective tissue cells wander into the coagulation tissue. It is a direct transformation of the fibrin clot and is identical with the process which was previously found (loc. cit.) to take place in the fibrin clots in living cultures of adult frog tissues and which has been noted above. The connective tissue cells which wander into the newly formed fibrous tissue from the cut edges do not digest the fibers but move among them and evidently cause a breaking up of the larger bundles of fibers into smaller ones and later we have formed, as a result, a tissue which is typical in appearance with regular connective tissue. The connective tissue cells are, in many cases, rounded cells when they first appear in the fibrous tissue but later they assume the typical elongated spindle shape of fibroblast cells. This change in shape appears to be due to the action of the cells in stretching along the fibers. The preparations do not show any connection between these spindle shaped cells and the fibers which had already formed, nor is there any evidence of an attempt by them to form new fibers intracellularly.

4. The staining reactions of the new fibrous tissue appear to be identical with the staining reactions of the connective tissue in frog skin. However the newly formed fibrous tissue can be digested in pancreatin and in this reaction it differs from the connective tissue in the skin of the adult frog. On the other hand extensive experiments with pancreatin on embryonic but fully formed connective tissue, obtained from the tail and skin of tadpoles of various ages, show that the pancreatin will digest it just as it does the newly formed fibrous tissue.

5. The experiments do not offer any evidence that the fibrous tissue formed by a direct transformation of the fibrin net is ever replaced by a connective tissue formed by an intracellular action. The newly formed fibrous tissue also appears to be identical in appearance, structure and function with regular collaginous connective tisue. Digestion with pancreatin will differentiate between the new tissue and adult connective tissue from the skin of the frog but this test fails to differentiate between the new tissue and embryonic connective tissue.

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17. *The effects of light on the retina of the turtle and of the lizard.* SAMUEL R. DETWILER, Osborn Zoological Laboratory, Yale University. (Introduced by R. G. Harrison.)

Although the effects of light on the Reptilian retina have been studied by many investigators, differences of opinion still exist as to the results obtained. According to most investigators (see Garten) no migration of the epithelial pigment takes place, nor do the cones contract. The present investigation was taken up with the hope of finally ascertaining whether or not light had these effects on the retina of reptiles. Coincident with this work it was found that the anatomy of the retinae studied differed in some respects from that of other forms and it was therefore necessary to make an anatomical study which might serve as a basis for physiological experiments.

If the form of the outer segment of the visual elements be taken as the criterion in determining the differences between rods and cones, then it can be said that the retinae of the turtles, Chelopus guttatus, Chelopus insculptus and Chrysemys picta and of the lizard, Sceloporus undulatus, contain no rods. Both double and single cones occur.

Garten's explanation of why the pigment migrates and the cones contract in light necessitates the assumption that, in a retina in which the visual elements are all of one kind, these reactions do not take place. Nevertheless light brings about not only a migration of the retinal pigment but a shortening of the cones in both the turtle and lizard. When the eyes of animals which have been placed in bright sunlight for 6 hours are compared with those of animals kept in darkness for 24 hours it is found that light causes a forward migration of the pigment in the turtle (Chrysemys picta) on the average of 3.6μ and in the lizard (Sceloporus undulatus) of 3.1μ. In addition to this effect on the pigment it is found that light causes a contraction of the cones to the extent of 2.3μ (based on a series of 10 measurements) in the turtle retina as well as a flattening of the pigment epithelial cells of 2.7μ.

Not only are these changes brought about in normal eyes, but also upon eyes the optic nerves of which have previously been severed. In such cases the pigment in the light eyes is 2.5μ nearer the external limiting membrane than in the light eye with the nerve intact, but this does not represent a greater relative amount of migration. The pigment in the dark eye in which the optic nerve is cut is 4μ nearer the external limiting membrane than in a dark eye with the nerve intact. A series of measurements show that the migration caused by light in eyes with the optic nerves cut is on the average of 2μ in contrast to a migration of 3.6μ in normal light eyes.

In addition to these effects, light is also found to reduce the amount of chromatin and Nissl substance in the ganglion cells so that they stain less darkly and more diffusely. It also decreases slightly the ability of the outer nuclei to take on stain, but, while it does cause them to lengthen slightly, it has no effect on the form and volume of the ganglion cells.

Attempts to see whether the pigment could be induced to undergo a greater migration by means of stimuli other than light were carried out by stimulating with currents, both interrupted and constant. Stimulation of the enucleated bulbus with an interrupted current of moderate strength brings about a position of the pigment approximately equal to that produced by light i.e., a migration of about 3.6μ.

Constant currents of 18 M.A. passed through the eye either in a centrifugal or centripetal direction for a period of 15 minutes cause a migration of approximately 2μ more than that produced by light. It also results in a considerable elongation of the cone myoid.

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A PRELIMINARY REPORT ON FURTHER EXPERIMENTS IN INHERITANCE AND DETERMINATION OF SEX

By Richard Goldschmidt
(Of the Kaiser Wilhelm Institut für Biologie, Berlin)
OSBORN ZOOLOGICAL LABORATORY, YALE UNIVERSITY

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In two former papers[1] the interesting 'gynandromorphism' produced by crossing the European and the Japanese races of the gipsy-moth (Lymantria dispar) was described and an experimental analysis of the phenomenon was attempted. From the data obtained important conclusions on the sex-problem in general were drawn. Although the main points seemed to be clear, a series of questions still remained open. One of them was, that the (apparently) same kind of crosses did not give the same results, if the material used had a different origin. It could be regarded as practically certain that the chief result, viz., the appearance of gynandromorphism in certain crosses found its right explanation in the hypothesis of a quantitatively different behavior or a different potency of the male sex-factors in the different races. Some of the experiments led me to suspect that this potency varied with the geographical distribution of the moth. Therefore it was one of my aims during a sojourn in Japan, to study the behavior of different local forms in that country in various crosses *inter se* and with different European forms.[2] These experiments are in no way to be regarded as completed, but the results so far obtained are so interesting and seem to bring the definitive solution of the problem so near, that a preliminary report on a part of them may be made.

A few words are first needed about the terminology. In previous papers I have used the term of gynandromorphism to indicate the sexual abnormalities produced in the racial crosses of these moths. It seems, however, no longer advisable to use this term, as it is applied more or less generally to quite a different phenomenon, *i.e.*, to individuals showing a mosaic of the characters of both sexes. In such a gynandromorph—see for example Boveri's late analysis of the Eugster bees[3]—a given organ or complex of cells is either male or female. But this is not the case with my moths; the entire individual represents a definite, quantitatively fixed stage intermediate between the two sexes. If we represent femaleness and maleness as the end points of a series, say one as 0 and the other as 100, a given specimen of these 'gynandromorphic' moths would be represented by the points 12 or 35, etc. These animals do not represent a mixture of the characters of the two sexes, but a definite point between the two extremes, maleness and femaleness. In the former papers some characters were thought to be a mosaic, viz., the color of the wings, which shows in certain stages definite patches of male and female design. It is now clear that this is but a consequence from the physiology of pigmentation, a fact that later will be the starting point for important theoretical discussions. So it seems advisable to introduce a new term to designate the phenomenon treated in these experiments. I shall call in the future the different sexual intermediates *intersexes;* female intersexes, if they are genetically females, but transformed to some stage towards maleness, and male intersexes if the transformation goes in the opposite direction; the whole phenomenon being called intersexualism.[4]

Before describing the new results a short account might be given of the different stages of intersexualism so far produced in the experiments. Every single step has now been bred from a normal female through the different grades of female intersexes to a normal male; also the steps starting from the normal male through male intersexes towards the female up to three-fourths of the way. Every single one of these steps can be produced now at will by crossing the right combination of races. Female intersexualism begins with animals, which show feathered antennae of medium size (feathered antennae are a male character) but which are otherwise entirely female in appearance except that they produce a smaller number of eggs, which are fertilized normally. In the next stage patches of the brown male pigment appear on the white female wings, in steadily increasing quantity. The instincts are still female, the males are attracted and copulate. But the characteristic egg sponge laid by the animal contains nothing but anal hairs, in spite of the fact that the abdomen is filled with ripe eggs. In the next stage whole sections of the wings show male coloration, with cuneiform female sectors between, the abdomen becomes smaller, contains fewer ripe eggs, the instincts are only slightly female, the males are attracted very little, and reproduction is impossible. In the next stage the male pigment covers practically the whole wing, the abdomen is almost male, but still contains ovaries with a few ripe eggs, the instincts are intermediate between males and females. Then follow very male-like animals, which still show in different organs their female origin and have rudimentary ovaries. They lead to practically male-like animals, called 'female-males' in my former paper, containing gonads which exhibit all transitional stages from an ovary to a testis. The end of the series is formed by males, which show in some minor characters, such as the shape of wings, still some traces of their female origin.

The series of the male intersexes starts with males showing a few white female spots on their wings. These become larger and larger, the amount of brown pigment correspondingly decreasing, finally (in extreme specimens) leaving only a few granules along the veins. Hand in hand with this the abdomen increases in size, reaching in the most extreme case two-thirds of the female size (without containing eggs!). The same is true for the instincts, which become more and more female. Further, the copulatory organs, which show in the female intersexes also a complete series of gradations from femaleness to maleness, and finally in the testis, which shows the first steps of transformation into an ovary.

There is another new point to be mentioned in this connection, as the fact that it was unknown is probably responsible for some errors in my former papers. It has been noticed that there are Japanese races, which contain a wing-pigmentation factor in the female sex; and the effect produced can be increased in certain crosses. In this way females with pigmented wings may arise, which may be mistaken for intersexes, and if this phenomenon occurs together with slight intersexualism, low grade intersexes may be mistaken for high grade ones. The further investigation of this will be of importance.

Going over now to the breeding results, we shall describe first only those relating to the female intersexes. It was found that there are in the European as well as in the Japanese forms, definite races possessing a special potency of the male sex-factors. If we cross forms of like or similar potency, the offspring is normal. But if we cross races with different potency of the male factors we get in F_1 female intersexes, provided the mother belongs to the race of lower potency. The degree of intersexuality depends of course upon the difference of the potencies. The European races so far known to me all show low potency; and in the Japanese races all grades are found from the lowest to the highest potency of those factors. If now any European race is crossed in any direction with one of the 'weak' Japanese races (the races K, Fu, M, H of my records) only normal females appear in the offspring. The same naturally holds true for crosses of any of these forms *inter se*. But all European females and also the females of the weak Japanese races, crossed with males of the 'strong' Japanese races (*i.e.*, G, O, A) ought to yield female intersexes, the grade of intersexuality depending upon both races involved in the cross. In detail the main results are the following; the F_1 males being left out of account:

1. All possible F_1 combinations between the weak Europeans S and F and the weak Japanese K, Fu, M, H contain only normal females.

2. Males of the moderately strong Japanese race G crossed with the very weak European females F produce nothing but high grade female intersexes ranging almost up to the above quoted 'female-males.' The same males produce, if crossed with the somewhat less weak European race S, nothing but medium female intersexes. The same males again crossed with the still less weak Japanese race H give in F_1 low grade female intersexes; and finally from the cross between these males and the highest of the weak Japanese races, K, we get only slight female intersexualism. There is to be added that the intersexuality affects every single individual that is genetically (one X-chromosome) a female.

3. Another of the Japanese races, which has a higher potency than G, produces in similar crosses with the weak European races the highest grade of female intersexuality, the so-called 'female-males.'

4. If the males used in the crosses belong to the strongest known Japanese races A and O (there is only a slight difference between these two which may be neglected here) and the females to the weak European races F and S, or the Japanese race H, all individuals genetically being females become transformed entirely into males. It may be added here that all results are based on fair numbers. For instance for this cross there are about 14 cultures with over 1000 individuals. In many cultures mortality is exactly recorded.

5. The following is to be regarded as an *experimentum crucis* for the correctness of the explanation given above: The Japanese race K proved to have a low potency of the male sex-factors, as crosses with European females produced normal offspring. Furthermore the same race K proved to be somewhat higher in potency than the European races by producing only the slightest degree of intersexuality in crosses with other males of the medium strong race G, whereas the European races gave in the corresponding cross with G-males up to high-grade intersexes. These same Europeans produce in the crosses with the strong races A and O nothing but males. Here we have an equation from which we should expect that in a cross between females from the races K and males A or O medium to high-grade intersexes will be produced. This is the actual result.

6. To these results may be added the following details:

a. The results are typical and, so far, without exception. They seem to be independent of external conditions as the same results were obtained identically (besides my former experiments carried on in Munich) in my cultures at the Bussey Institution, in Boston[6] and those of my assistant Mr. Seiler in Berlin.

b. The grade of intersexuality in one culture is subject to a regular and continuous variation around a mean. If we divide the distance between femaleness and maleness in 100 grades a given culture would show a symmetric variation of a certain range, say 20 grades, around a mean of 50 or 60, etc. What causes certain differences in the position of the modal class in sister cultures is not yet clear, but will be very important for the theoretical side of the question. It is to be expected that at the two end points of the series the individuals overlap on the normal, *i.e.*, respectively normal females and males. This was actually found in the border cases. If all individuals which are genetically females are changed into males some minus individuals may be recognizable by their somewhat female shape of the wings, proving that the variability extends into the normal, too. It is to be hoped that the statistical treatment of these facts, together with the F_2 results and the data regarding the male intersexes will open a way to an exact calculation of the relative values of potency.

c. In the crosses producing nothing but males, occasionally there appears a single normal female, hatching as the last individual of a given culture. It is very probable that we face here another case of non-disjunction (Bridges). A spermatozoon without X-chromosome ought to give a normal female with every egg (assuming naturally that the male factors are carried in the X-chromosomes). So far I have succeeded only in finding a single spermatocyte II with 30 instead of 31 chromosomes.

So far only the facts regarding the female intersexes have been recorded, and they form a strong support for the views of my earlier papers in regard to the sex-problem. But the new results about the male intersexes show that in one important point the hypotheses have to be changed. I stated there that the male intersexes appear in F_2 from the reciprocal cross, not giving female intersexes in F_1. The fact that exactly one-eighth of the males were intersexual corroborated strongly the Mendelian formula of sex-inheritance used by me, working with two pairs of sex factors. The new results give a different aspect to the facts:

1. The appearance of the male intersexes in the said crosses is a single event conditioned by the two races involved in the cross. The ratio $\frac{1}{8}$ is typical, too, only for the special combination. In other crosses any ratio between 0 and 50 percent may be typical.

2. A new fact of decisive importance is that male intersexes may be produced in F_1. So there were a few individuals in the crosses female K × male S and female O × male H. And in the cross: weak Japanese female K × weak Japanese male H, nothing but intersexual males appear in F_1.[6] These facts are of greatest importance for the whole question. Together with some other F_2 results and the insight in some former errors of interpretation they make it very probable that the female part of the formulae F F M m = ♀

F F M M = ♂ does not mendelize but is inherited plasmatically. This part too shows different potencies and the final result is the combined effect of both groups. However, it seems undesirable to insist on this point, as the decisive experiments, which may bring the complete solution, have not yet been accomplished.

[1] Goldschmidt, R., Erblichkeitsstudien an Schmetterlingen. I., *Zs. induct. Abstammungslehre.*, **7**, (1912); and Goldschmidt, R., und Poppelbaum, H., idem. II. *Ibid.*, **11** (1914)

[2] The existence of such local forms could be shown by breeding experiments. The problem of the geographical races of this moth has been for many years the object of my principal studies. However, the results are not yet ripe for publication.

[3] Boveri, Th., Ueber die Entstehung der Eugsterschen Zwitterbienen., *Arch. Entw-Meck., Leipzig*, **41** (1915).

[4] The fact that male and female intersexes are different calls certainly for an explanation, but will not be discussed here. It may only be said that it is a question of the physiology of development.

[5] I wish to express my sincerest thanks to Professor Wheeler and all the members of the staff of the Bussey Institution, Harvard University, for their kindness in granting me the facilities for prosecuting my work.

[6] A similar cross with the same race H but another mother has been carried out already with the same result by Toyama's assistant, Dr. Machida, to whom therefore belongs the priority of this discovery. I am indebted to him, too, for the material of the race H. I do not know whether he has meanwhile published this result.

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GENETIC FACTORS AND ENZYME REACTION

IN spite of the great progress in the knowledge of facts in genetics the nature of genetic factors may well be regarded as unknown. Various theories have been proposed but only a few steps have been made to attack the problem experimentally. Those who approached it from the physiological-chemical side all seem to agree that the unit-factors are to be compared in some way to enzymes (Loeb, Robertson, Moore, Bateson, Riddle, etc.) or expressed more generally "that the hereditary factor . . . is a determiner for a given mass of certain ferments" (Loeb and Chamberlain, 1915).[1] At first sight there are not many ways visible of an experimental attack on this problem. One is described by Loeb and Chamberlain in the following words:

> If we wish to carry this view (with which we sympathize) beyond the limit of a vague statement, we must either try to establish the nature of these compounds by the methods of the organic chemist, or we must use the methods of general or physical chemistry and try to find numerical relations by which we can identify the quantities of the reacting masses or the ratio in which they combine.

Some steps in this direction have been made by Loeb, Robertson and Ostwald, who tried to prove that the phenomena of growth may be understood as autocatalytic reactions; by Moore, who compared the velocity of development of a dominant character in homozygotes and heterozygotes; by Loeb and Chamberlain, who followed the more indirect way of proving the enzyme-reaction-like basis of a certain kind of fluctuating variability. It is further known that Miss Wheldale and Keeble are approaching the question by a direct study of the chemistry of plant pigments in hybrids of known constitution and quite recently a very interesting paper on hair-pigments in rodents has been published by Onslow.[2]

For some time I have had similar ideas in regard to these questions in connection with genetical experiments, approaching the subject from quite an unexpected side. It was not the intention to publish them before the entire work was finished. But as this will take some years longer and the subject is becoming meanwhile more popular, it might be advisable briefly to point out the ways in which I reached conclusions very similar to those of Loeb, etc.

The genetical reaction which is concerned primarily in my experiments is the pigmentation of the wings of moth. Its dependence upon genetic factors is well known and its chemical character—the amino-acid-oxydase reaction—is comparatively clear. In one set of experiments it could be shown how the quantity of pigment formation depends upon the quantitative combination of the hereditary factors.[3] The experiments were started in 1909 with the purpose of working out the genetics of melanism in moths. The experiments are so far finished, but details about them can not be published, because the records are not available just now. But one point can be stated in a general way. In my example, the nun (*Lymantria monacha*), all gradations are found between a white animal with the characteristic zig-zag bands and a completely black one. The breeding experiments show that these intermediates are to be explained by combinations of some, partly sex-linked, factors for pigmentation. The comparison of the wings shows that the pigmentation starts from certain points of outlet and spreads thence over the wing, gradually encroaching upon the white scales. Obviously there corresponds to every combination of factors, an enzyme reaction, definite in quantity. Of course, the same conclusion could already have been drawn from Nilsson-Ehle's well-known studies on oats. But the meaning of the reaction is so much more evident in the insect case.

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[1] *Jour. Exp. Zool.*, Vol. 19, 1915.

[2] *Proc. R. Soc. S. B.*, Vol. 89, 1915.

[3] Onslow's results are in the same line.

The other way which led to similar conclusions in regard to the connection of hereditary factors with the quantity of enzyme reaction is quite an unexpected one. In some previous papers I have published the results of experiments in determination of sex in the gypsy moth and a report upon their further progress is now in press in the *Proc. Nat. Acad. Sc.* The point which concerns us here is the following. We have found a series of races of that moth, which differ in regard to the quantitative behavior of their sex-factors. We could prove that in a cross between these races the resulting sex with all the secondary sex-characters depends upon the quantitative relation of the male and female set of sex factors. In the hybrids all kinds of combinations of these two sets varying in their relative quantity, can be brought together. And the result is that every single step between the two sexes, for which I proposed the term intersexes, may be produced. The external characters of these animals now are determined in the following way: the female factorial set would produce entirely female characters, and in the same way the male set male characters. The real effect is a function of the arithmetical difference of these two. If this difference is in favor of one or both above a certain quantity, say $f - m > x$ or $m - f > z$, the pure sex is produced. But if the difference is beyond the constant minimum z and x, an intersex is produced. And the quantity of intersexuality increases proportionally to the decrease of the values $f - m$ or $m - f$. The effect of such a competition of two sets of factors, both influencing the same characters in different directions, is, of course, the same as if only one factor of a variable quantitative efficiency were present. And now we are able to draw a parallel between the quantities of the hereditary factor and the quantities of the observed enzymatic reaction causing the coloration of the wing.

In the colors and markings of the wings of these moths at least four factors or sets of factors are involved, as is shown by loss-mutations. The normal females have white wings with transverse zig-zag bands, and, in addition, a crescent-shaped spot and a point near it, resembling the Turkish emblem (crescent and star). In the males the same markings are present and also a diffuse color covering the entire wing and varying from light gray to almost black in different races. In a mutation, which appeared some years ago in my cultures, all ziz-zag bands, except the one near the edge of the wing, disappeared. The mutation is not sex-limited and independent of the general color of the wing as is shown by breeding tests. This general color is again subject to mutations in the male; and there appeared another mutation also in which the sex-linkage is broken and the female wings are colored. The following remarks apply only to the normal, general wing-pigmentation, linked with the male sex.

It is known through the work of Federley and others that this pigment flows out from the wing-veins spreading over the entire wing. And it might not be unsafe to say that it is the oxydase which diffuses from the hemolymph in the veins into the scales. If we now study the different grades of intersexuality produced in our experiments, we realize that every step leading from a normal female through the different grades of intersexes to a male, or, vice versa, from a male to the female, is characterized by a definite intermediate step of wing-pigmentation. The color of the pigment is constant but its quantity is variable. And one sees at first sight that in the different intersexes a certain amount of pigment-producing oxydase, parallel to the quantitative behavior of the sex factors, is furnished by the veins, varying from 0 per cent. in the female to 100 per cent. in the male. If a male is becoming intersexual, white cunei appear between the veins on the brown wing. Their position and shape is irregular. The total unpigmented area in different animals of the same constitution, is, however, approximately the same. With growing intersexuality—as measured by all organs of the animal—the white spots become larger. And an inspection of the wings shows immediately that there must be present an amount of pigment or, more correctly, of oxydase, quantitatively fixed, and corresponding to the quantitative value of $m - f$; and that the given quantity (or concentration) flows out from the veins over the wing, producing brown scales, whereever it happens to come. With increasing inter-sexuality the phenomenon becomes still clearer. A stage is reached, where a white wing shows brown, pigmented venation; in some places a short stream of pigment seems to flow out from a vein. In still more advanced intersexual males, about two thirds transformed into females, only a few pigment spots and stripes are to be found on the wings along the veins. In the female intersexes the opposite process is observed, but the details are somewhat different, showing that these depend upon the genetically given wing structure, different in both sexes.

It seems that this case is an exceedingly clear one, demonstrating the principle ad oculos. But it may be of even greater significance. All organs different in the two sexes are affected in some way by the intersexuality. There is some hope that it might be possible to obtain by their analysis a similar insight in the process of growth, localization, symmetry, etc., involved in morphogenesis. But I think that it is already clear from the foregoing remarks, that we are right, when we reached, independently, the conclusion that the hereditary factor is a determiner for a given mass of ferments; and we can demonstrate it by the fact that a quantitative difference in the potency of hereditary factors causes a parallel, quantitatively different, enzyme production.

RICHARD GOLDSCHMIDT
OSBORNE ZOOLOGICAL LABORATORY,
YALE UNIVERSITY,
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20. *On the reversal of laterality in the limbs of Amblystoma embryos.*

Ross G. Harrison, Osborn Zoological Laboratory, Yale University.

For the purpose of investigating certain factors concerned in the development of paired limbs a series of experiments have been made upon Amblystoma embryos. That portion of the body wall from which the fore limb normally develops was excised before differentiation had begun, and the disc-shaped piece thus obtained and consisting of ectoderm and mesoderm, was transplanted to another embryo. The results were as follows:

1. A limb bud from one side of the body implanted in the place of a bud previously removed on the opposite side, develops in a large proportion of cases (over one-third) into a limb of the side to which it has been transplanted, i.e., its laterality is reversed. The limb thus grown is in most cases perfect in form and function, though it is usually somewhat retarded in development as compared with the normal non-transplanted bud. Perfect regulation of the transplanted part with respect to the organism as a whole is thus brought about. In other cases (almost one-third) duplicate limbs arise, the twins being of opposite laterality. Observation of many of the cases in which single normal limbs develop shows that in early stages the bud tends to grow out as of the side to which it originally belonged, i.e., not reversed; then a reduplicating bud appears and this soon gains the upper hand, ultimately becoming the limb with reversed laterality, while the original is resorbed or in some cases reduced to a small spur which may remain permanently attached to the fully developed limb. In a few cases a single limb of original laterality develops though none of these have been found perfect. In the remaining cases the limbs are either rudimentary or the transplanted tissue is entirely resorbed.

2. If the limb bud from one side of the body is transplanted to the opposite side and placed not in normal position but on the side of the body between the fore and hind limb, the results are very different. A much larger proportion (over half) develop very imperfectly or are absorbed, about one-quarter develop with their original laterality and some reduplicated appendages may arise. In but one case has the laterality of the single limb been reversed.

3. In cases where the limb bud is implanted in normal location on the same side but turned upside down, about one-third of them develop into normal limbs in normal position. About one-sixth give rise to twin limbs and the remainder are mostly imperfect or are resorbed. Observation of the development in individual cases shows that the single limbs gradually acquire their normal orientation by rotation.

4. Limb buds placed upside down on the same side of the body between the fore and hind limb develop in one-half of the cases into limbs of opposite laterality. In no case has a single limb of the original laterality been produced and in only one case a pair.

5. Experiments in which the limb bud was transplanted to the same side of the body in normal position all resulted in normal development,

showing that the results of the previous experiments are due to the abnormal relations of the transplanted rudiment with respect to its surroundings, and not to the operation as such.

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33. *The germ cell cycle in the mouse.* W. B. Kirkham, Osborn Zoological Laboratory, Yale University, introduced by R. G. Harrison.

The material for this research is (1) a complete series of embryos, at about 24 hour intervals, from the fertilization of the egg to the time of birth, and (2) a complete series of gonads of both sexes, likewise representing 24 hour intervals, and covering the period from birth to 50 days old.

The primordial germ cells have not been found in embryos younger than 11 days, but from that time on their development has been traced without a break. They are characterized by having large, round nuclei, and clearly defined cell membranes, while the adjacent epithelial cells have smaller, oval nuclei, and indistinct membranes.

Mouse embryos of 11 days show the primordial germ cells scattered through a much larger number of epithelial cells, without sexual differentiation. At 13 days testes can be distinguished from ovaries by the evidence in the form of tubule formation. In male embryos of 15 days it is evident that the primordial germ cells form the cores, while epithelial cells line the inner walls of the tubules, and this arrangement assumes great significance in view of the fact that older male embryos show degeneration of the primordial germ cells, and a simultaneous increase in size, together with a rounding up of outline, on the part of the nuclei of the peripheral epithelial cells, which can now be called spermatogonia. Some of the primordial germ cells in male gonads divide after tubules are formed, but all appear sooner or later to degenerate, and about 8 days after birth none are longer visible.

The development of spermatozoa from the spermatogonia goes on at about the following rate, variations of 2 or 3 days being allowable in each case to cover individual differences: the first primary spermatocytes appear on the thirteenth day after birth; first secondary spermatocytes on the twenty-third day; first spermatids twenty-sixth day; and first spermatozoa, attached still to Sertoli cells, on the thirtieth day. Not until the thirty-eighth day after birth have spermatozoa been found in the seminal vesicles, and then in only small numbers.

No new evidence has been found as to the origin of the Sertoli cells, nor can any definite statement be made as to the time of their first appearance in the testes. In testes of 30-day old males Sertoli cells are present, and they can easily be found in any older specimens.

The female gonads during the embryonic period show, in contrast to those of the male, no degenerating cells, but instead a constant increase in size and in number (by mitosis) of the primordial germ cells. At the time of birth the largest primordial germ cells already possess a single enveloping layer of epithelial cells, and can properly be termed oogonia.

After birth the primordial germ cells of the female, or oogonia, increase still further in size, but no increase in number takes place. Many layers of epithelial cells arrange themselves around the largest oogonia, lacunae appear inside the follicles thus formed, and it would seem on the seventeenth day after birth as though ovulation was about to occur. However, instead of follicles rupturing at this early age, from about the seventeenth to the fortieth day the oogonia which have grown to full size degenerate within their follicles, the first indication being the chromatolysis of the follicle cells, which may or may not be followed by the maturation or fragmentation of the egg. In no instance has a normal maturation spindle or polar body been seen in this material.

The stimulus which causes ovulation first comes at about 40 days after birth, as the earliest ruptured follicles in this material are in ovaries from a 40-day old female, but a female of 50 days was found to contain embryos which correspond with the usual development at 12 days, while another female whose age was 48 days possessed ovaries with no ruptured follicles.

In brief this investigation shows that the sexual cycle in the mouse is completed in about 60 days, but that the male and female germ cells come from different cell lines, the oogonia being direct descendants of primordial germ cells, while the spermatogonia arise, not from primordial germ cells, but from what appear to be epithelial cells.

34. The prolonged gestation period in nursing mice. W. B. Kirkham, Osborn Zoological Laboratory, Yale University, introduced by R. G. Harrison.

This is an embryological investigation of the discovery published by Prof. J. F. Daniel in 1910 that among mice, nursing females if again fertilized give birth to a new litter only after a longer gestation period than that of non-nursing females. He further stated that the delay in parturition was equivalent to approximately one day for each young mouse nursed.

Two series of embryos have been studied, one taken from nursing, the other from non-nursing females. Both series are spaced at 24 hour intervals, and cover the period from the fertilization of the eggs to the time of birth.

The differential characteristics of each day's development having been determined in the series from non-nursing females, a comparison with the nursing series shows the following facts:

(a) Ovulation and fertilization, when they occur, occur at the same time relative to parturition,—namely the following night,—in both nursing and non-nursing females, although nursing females are much less liable to ovulate.

(b) Segmentation of the fertilized eggs and their passage down the Fallopian tubes take place at the same rate in both nursing and non-nursing females, resulting in blastulae in the lumen of the uterus on the fourth day.

(c) Implantation occurs in non-nursing females during the fourth day, while in nursing females implantation does not take place until the close of the twelfth day. This delay would appear to be connected with the fact that up to this time the nursing young are obtaining all or most of their nourishment from the females.

(d) The stages following implantation show progressive development in non-nursing females, but in nursing animals the time elapsed since fertilization bears no fixed relation to the stage of development of the embryos, nor does this appear to be associated with the number nursing nor with the total number of embryos. It can be stated, however, that almost every set of embryos examined is behind the stage of development it should have reached if growth and differentiation at the rate of embryos in non-nursing animals were to bring about birth at the date set by Daniel's formula, a day's delay for each one nursing.

The prolongation of the gestation period in nursing mice is therefore largely due to delayed implantation.

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41. Artificial parthenogenesis in Cumingia. MARGARET MORRIS, Osborn Zoological Laboratory, Yale University. (Introduced by R. G. Harrison.)

The eggs of the mollusc Cumingia tellionoides can be made to develop parthenogenetically by the use of heat followed by hypertonic sea-water. Some of the eggs so treated form polar bodies in a normal manner, but others pass at once to a two-cell stage without undergoing maturation. Experiments were made which show that the eggs in which maturation has been suppressed may develop to fairly normal cellular larvae, while those which have formed polar bodies do not do so.

It is seen from a study of preserved material that in some eggs the chromosomes of the first maturation spindle divide normally giving rise to two daughter nuclei which are both retained in the egg, which has, therefore, the full amount of chromatin. The cytological study of the later stages of these eggs has not yet been completed, but it seems certain that they give rise to the swimming larvae.

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45. Some phases of cell mechanics. THEOPHILUS S. PAINTER, Osborn Zoological Laboratory, Yale University. (Introduced by R. G. Harrison.)

In a series of studies an attempt is being made to analyze some of the forces at play within the cell, along the general lines begun by Boveri and by Wilson. The material used has been the sea urchin egg and the method of approach has been: first, to produce monasters in the eggs and then to follow, in the living and in the sectioned egg, the changes through which the single aster goes. .Second, to treat the eggs with narcotics such as will inhibit the cytoplasmic radiations and then to follow the centrosomes, chromosomes, etc., in their behavior. Several points of general interest will be touched upon here.

1. Under certain conditions, "spiral asters" appear in the monaster eggs. The conclusion reached after a detailed study of eggs of this type is, that the bending of the aster fibers is brought about by movements in the cytoplasm lying outside of the centrosphere. This interpretation gives a satisfactory explanation for the spiral asters observed by various workers, in Nemerteans, Annelids, Molluscs, and in Vertebrates. A second conclusion, drawn from the study of spiral asters is, that the shifting of the spindle observed in eggs, especially during polar body formation, is due to movements in the cytoplasm.

2. Observations on the living monaster eggs has shown that when the aster retreats to one side of the egg (as in normal divisions) there is a flow of the superficial protoplasm towards the opposite side of the egg. Thus, in Arbacia, nearly all of the pigment is swept to one pole, the side opposite the aster. Bütschli attributed great importance to this movement in cell division.

3. When eggs are treated with chemicals which suppress the cytoplasmic radiations, a spindle is formed within the boundary of the nucleus, the chromosomes divide and the centrosomes separate. Cytoplasmic division, however, does not follow. Thus it is clear that the cytoplasmic radiations do not pull the centrosomes apart, as has been thought by many workers. They are probably concerned with cytoplasmic cleavage.

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54. *A disguised type of smooth muscle cell.* R. A. SPAETH. Osborn Zoölogical Laboratory, Yale University. (Introduced by R. G. Harrison).*

The melanophores of the lower vertebrates—fish, amphibia and reptiles—are described by most authors as modified connective tissue cells. The evidence for such a conception rests chiefly upon a morphological basis. Leydig showed that no sharp morphological distinction exists between unpigmented connected tissue cells, inactive, pigmented connective tissue cells and chromatophores (melanophores) containing actively migrating pigment granules.

Morpholgical characteristics alone are not, however, sufficient finally to classify any type of cell. Little evidence is to be adduced from the embryological history of the melanophore concerning its ultimate nature, since both connective tissue cells and melanophores develop from wandering mesenchyme cells. However, a consideration of the physiological responses of the melanophore shows that it bears a far closer resemblance to certain types of smooth muscle cells, e.g., those of the sphincter pupillae, the digestive tract, and the radial elements of the cephalopod chromatophores, than to any type of connective tissue cell. Thus, for example, chemical, electrical and thermal stimuli which produce contractions in these three types of smooth muscle also bring about contractions in the melanophore. Appropriate treatment with $PaCl_2$ initiates a slow pulsation in the melanophore. A study of the rate of rythmic contraction shows it to be of the same order as the spontaneous contractions of excised portions of the digestive tract.

The work of Chun has shown conclusively that the chromatophores of cephalopods develop from single smooth muscle cells by a complicated metamorphosis. Thus the establishing of the vertebrate melanophore as a modified smooth muscle cell—a view previously suggested by Steinach, Franz and Hoffman and formerly questioned by me, is of particular significance, since it reduces the color-changing mechanisms of both vertebrates and invertebrates to a common basis, i.e., a specialized type of smooth muscle cell.

In the melanophore, the motor function of the smooth muscle cell is lost and there is developed a modified motility in the migration of pigment granules, which may serve as a means of protecting the animal. An analogous case in that of the electric organ in fish where a striated muscle cell loses its motor function completely, and the action current, normally of relative insignificance, becomes a physiological end in itself and a formidable means of protection.

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62. Blastolysis as a morphogenetic factor in the development of monsters. (Lantern.) E. I. WERBER, Osborn Zoological Laboratory, Yale University.

On a previous occasion I have reported some experimental results of the action of products of pathologic metabolism on the developing egg of *Fundulus heteroclitus*. It was then shown that a very great variety of monsters, similar to human and other mammalian monsters, can be produced with solutions of, particularly, two substances which are known to be present in the blood or urine of individuals suffering from disturbances of carbohydrate metabolism, namely, with butyric acid and acetone.

Although in each experiment a great diversity of morphological results has been obtained by the employment of the same method, it seems reasonable to conclude that at least some factors are common to the morphogenesis of all monsters resulting from a given experiment. A study of many deformed embryos from butyric acid and acetone solutions and analysis of existing morphological relations has, indeed, convinced me that in nearly all malformed embryos of my experiments evidence can be found of a common morphogenetic principle. For, in nearly all of them the action of some forces which tended to dissociate and destroy the germ's substance is apparent.

This action is, however, a 'complex component' in the sense of Roux, as it is probably conditioned by a number of factors present in a varying degree in individual eggs of the same experiment Analysis of these factors will for some time to come be one of the objects of my investigations. A the present time it is the results of their collective action, to which latter I have applied the term blastolysis, that I wish to call your attention.

Blastolysis either destroys part or all of the germ's substance, or it may split off and disperse parts of the latter. The numerous meroplasts resulting from my experiments and particularly such teratomata as the 'solitary eye' and the 'isolated eye' bear ample witness to this process. The presence on the yolk of a small fragment of nervous tissue with an eye at a great distance from a malformed embryo, or even without an embryo, would, in my opinion, seem to permit of no other interpretation.

While, as we see, some effects of blastolytic action are thus strikingly evident on examination of the eggs or embryos *in toto*, other effects can be recognized only by a study of sections of them. The latter I regard as very important, for it often reveals conditions which have a significant bearing on the ontomechanics of the vertebrate organism. As we shall presently see, it also sheds light on the mode of formation of some terata, which has so far been the object of fruitless speculation.

The following are a few out of a great and constantly increasing number of facts which microscopic examination of malformed embryos may disclose. In the head region a lens may be found free, not associated with an eye, or a profusion of small lentoids may be met with, even though eyes may be entirely lacking. The complete absence of the spinal cord (amyelia) and of the notochord was noted in one embryo. That the latter condition is due to blastolytic elimination of the parts of the germ's substance which were eventually to form the spinal cord and notochord and not to an inhibition in the development of these parts would seem to be evidenced by the experience that both the spinal cord and the notochord are usually present in highly deformed embryos. Moreover, the notochord particularly, can not infrequently be found to be partially doubled—a condition indicating the action of a force which tends to dissociate parts of the early embryonic primordium (blastolysis).

Similarly, ectopia of some organs which may occasionally be found on microscopic examination of my experimental monsters would also seem to point decidedly to blastolytic action. Thus I have found in one embryo in sections through the anterior part of the head a tube-like structure which made the impression of a fragment of the intestine, while in other embryos a rudimentary eye anlage (optic vesicle) has been found on the ventral side of the posterior part of the brain, at, or beyond the level of the ear vesicles. Such findings indicate unmistakably that dissociated parts of the embryonic primordium sometimes become dislocated and are apparently capable of independent further development and differentiation in their new surroundings.

A beautiful illustration of such blastolytic dispersion of minute parts of the embryonic primordium is presented also by some monstra anophthalmica asymmetrica, on sections of which I was able to find posterior to the single lateral eye and ventrally from the brain a very small fully differentiated fragment of an optic cup. In the latter the chorioid coat and all layers of the retina including the rods and cones-layer are at the same stage of differentiation as these parts of the single eye. The bearing of these facts is evident. For they show us the nature of the formative cause underlying the condition of single-eyedness. Thus in the case of lateral single-eyedness the potential optic anlage has been elminated by blastolytic destruction, an event which occasionally may be betrayed by a dislocated optic cup fragment, the only remnant of the anlage of the lacking eye.

In the light of our theory of blastolysis, supported by these and many other facts, the *terata of the eye*, such as cyclopia, synophthalmia and monophthalmia asymmetrica *must*, as Spemann has rightly pointed out, *be regarded as due to a defect* and not to an inhibition as has been claimed by others and notably by Dareste and by Stockard. This view is further strengthened by the condition of the brain of teratophthalmic embryos. In asymmetrically monophthalmic embryos the brain is on the side, which lacks the eye, usually distorted in its relation to the chief body axis and sometimes a dorsol-ateral part of it on the eyeless side is seen to be lacking, just as if it had been mechanically removed.

In synophthalmic and some cyclopean embryos I have always found the forebrain to be exceedingly small and unilobed, while the rest of the brain may often be apparently normal. Here, evidently, the blastolytic defect involved the potential interocular area and parts of the potential ophthalmic anlagen.

The fused olfactory pits and the proboscis-shaped mouth of such embryos (the latter due to approximation and partial fusion of the potential maxillary and mandibular arches) furnish additional evidence for the correctness of the view according to which cyclopia or synophthalmia is due to a defect.

Some experiments with acetone in which solutions of low concentrations and long exposures have been employed, have yielded results which furnish further evidence for the blastolytic defect underlying the formation of ophthalmic terata. While some of the eggs so treated have given rise to synophthamic or monophthalmic monsters, many of them have developed into embryos with two large, greatly protruding eyes. Examination of sections shows that these eyes are elongate and ovoid in shape, and it is difficult to escape the impression that during their development some forces must have been acting which tended to disrupt or disintegrate them (blastolysis). The blastolytic defect, although of a moderate degree in these cases, is strikingly exhibited by the small and wanting forebrain. Owing to this defect the eyes appear in posterior sections to be approximated. No fusion of the eyes has, however, occurred, for the part of the potential interocular area eliminated by blastolysis has not been large enough to cause a fusion of the potential eye anlgen. It could not be imagined that this approximation of the eyes is due to an inhibition which prevented the brain from pushing out the eye vesicles forwards and lateralwards, for these eyes are in the usual lateral position in the head, from which they so grotesquely protrude. They certainly have been pushed out by the brain, and even more so than is normally the case. Their approximation is secondary and is solely due to blastolytic elimination of an intermediate part.

As we see, the morphogenesis of some deformities, like those of the eyes and of other terata finds a simple and rational explanation, if the principle of blastolysis is consistently applied in their analysis. More data in support of the correctness of this analysis and a full treatment of the subject will be found in forthcoming papers.

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THE EFFECTS OF THE REMOVAL OF THE NASAL PITS IN AMBLYSTOMA EMBRYOS

HAROLD SAXTON BURR

From the Osborn Zoölogical Laboratory, Yale University

FOURTEEN FIGURES (THREE PLATES)

INTRODUCTION

Many of the difficulties encountered in the study of the nervous system by the usual methods are obviated by experiments upon embryonic material in which nerve fibers are not yet developed and the blood has not begun to circulate. In this way it is possible to observe the effect of the removal of an end organ upon the central nervous system without undue complication of the factors involved, and furthermore such a method makes possible the study of the formative influence one part may exert on another during ontogeny.

The number of investigators who have applied this method is relatively small.

In 1906 Braus extirpated the forelimbs of the larvae of Bombinator before the outgrowth of the brachial plexus, with a view to determining the effect of the absence of the limb on the ventral horn of the spinal cord at the level of the brachial plexus. The experiment showed that there was at first no observable effect on the cord. The brachial nerves grew out into the surrounding tissue and ended more or less blindly. The size and number of the motor cells of the ventral horn was in no distinguishable manner changed. But this investigator found that when the operated larvae were kept alive until just before metamorphosis and then killed, a distinct reduction in the size of the cells of the ventral horn was discernible. Hence, he concluded, as a corollary to the theory proposed by Roux in 1885, that the development of the central nervous system was readily divisible into two periods, in the first of which growth and differentiation was independent of functional activity, but in the second of which functional activity determined whether or not growth would continue. But as Forel had previously pointed out, the atrophy which made the distinction in size of the cells evident was an exceedingly slow process as evidenced by the fact that this distinction did not occur until just before metamorphosis.

Two other investigators may be mentioned here, who have worked along this line. In 1909 Shorey performed a similar series of experiments on chick and amphibian embryos. She found in the chick an almost immediate effect of the destruction of the limb bud on the size of the ventral horn at the limb level. But whereas Braus concluded that the neurones were self-differentiating and that the reduction in size was a secondary one due to the absence of function, Shorey argued that the neurones did not differentiate without the stimulus of function and environment.

So far as it has been possible to ascertain, Dürken ('11) is the only investigator who has attempted to carry this question further to a consideration of the effect of the absence of an end organ on the gross morphology of the brain. He found very extensive changes in the shape and organization of the brain of Rana fusca and R. esculenta when the fore or hind limbs were extirpated at an early age. The abnormalities affected not only the somatic motor areas, but also parts of the diencephalon and telencephalon, notably in the roof. As will be seen, the experiments with which the present report is concerned show no such fundamental upheavals.

The last two named investigators, while using embryonic material, worked with individuals in which the peripheral nervous system had already partly developed, and it is suggested that the fundamental discrepancy between their results and those that follow may be due to this factor.

From the experiments of Braus it is evident that there is a possibility that Gudden's atrophy, when it can be induced, would make possible the tracing of a given system of nerve fibers from its peripheral ending to its cortical origin with all its collateral connections. The problem lies in making a lesion that will cause Gudden's atrophy, and then in keeping the animal alive sufficiently long for the atrophy to reach its height.

In the spring of 1911, Dr. R. G. Harrison suggested that the removal of the nasal placode of Amphibia would be a practical method to attack such problems. Frog larvae were first used for the experiments but as Bell showed in 1907 the placodes often regenerated, a fact which would destroy the value of the material for this study. Bell found this regeneration occurred in a large number of cases, even where it was believed the placode had been entirely removed. This regeneration may have been due to the fact that the regenerating area about the placode was not removed.

In Amblystoma, the placode can be easily extirpated. The nasal Anlage is readily distinguished from the surrounding tissue, and hence complete removal is a relatively simple matter.

In the course of the experiments it was found that the extirpation of the nasal placode made possible not only a study of the neurological problems involved, but also a part, at least, of the developmental mechanics of the skull. Since the material for this morphological work was kept alive for some months, a study of the reactions to food stimuli of the operated and normal forms was also undertaken. These three aspects of the problem form the body of the following report. Owing to the difficulty encountered in keeping operated forms alive through metamorphosis, the report of the histological changes in the telencephalon as a result of the operations must be deferred until later.

MATERIAL AND METHODS

Amblystoma larvae 5–6 mm. long, were used for all of the operations, of which two series were performed. In the first the nasal placode of the right side only was removed; in the second both were extirpated. The material obtained from the first series served as a basis for the morphological investigation—that from the second for the physiological experiments and as a check for the morphological study.

The operations were performed with a pair of iridectomy scissors under the binocular microscope. During the operation, and for the subsequent twenty-four hours, or until healing was accomplished, the embryos were kept in a 0.2–0.4 per cent salt solution. They were then removed to individual dishes in which the water was kept fresh. When the larvae were ready to feed they were removed to aquaria, balanced largely with Lemna and Ceratophyllum. Records were kept of the history of each operated specimen.

Care was taken in all of the operations not to injure the underlying forebrain, since any injury causes noticeable defects in the telencephalon.

In the series of experiments under consideration a large number of unilateral operations were performed. The material thus obtained was killed at frequent intervals, the oldest larvae being about six months old dating from the time of operation, and the youngest only a few hours old. The killing fluid used was sublimate acetic. Ehrlich's Haematoxylin and Congo-red were used to stain the 10 μ sections. Normal material was subjected to the same technique for controls. From the sections of the oldest larvae a wax reconstraction was made of the rostral part of the skull and brain by the Born method.

The experimental investigations that have up to the present time been reported on Amphibia, have tended to show that the Anlage of the nose readily regenerates when parts are removed. Bell ('07) goes so far as to assert that the complete extirpation of the nasal anlage in the frog does not prevent the regeneration of a nasal sac. In the few cases he reports, what be believed was complete extirpation resulted in every case in a regenerated sac, sometimes perfectly normal in size and structure, and in other instances, smaller than its fellow.

The present series of experiments started with the extirpation of the nasal placode of Amblystoma. It became evident at once that careful and complete extirpation of the rudiment was not followed by regeneration. Of the two hundred and thirty-two operations performed, only four showed any external evidences of a regenerating capsule. Later study by means of sections added two more to the number.

A number of experiments were then performed on the frog embryo to see if there was any difference in the behavior of the nasal anlage in two such closely allied forms. Great care was taken to remove all of the nasal placode. It was apparent from the outset that the placode of the frog is not nearly as compact nor so clearly differentiated from the surrounding ectoderm and not so easily separated from the underlying mesenchyme as it is in Amblystoma. Hence the complete extirpation is a much more difficult and delicate matter. External inspection showed twenty-two regenerating capsules out of seventy-six operations. Sections of a number of the remaining fifty-four taken at random showed an occasional abortive pit. It is evident then, that complete extirpation in the frog does preclude regeneration quite as completely as in Amblystoma. It is possible that here, as in the regeneration of the limbs, lens and gills, there is a circumscribed area about the anlagen which on the removal of the anlagen may regenerate it. Apparently in the frog this regenerating area is less restricted than in Amblystoma, if any such is present in the latter.

REACTIONS OF NORMAL AND OPERATED LARVAE

Turning now to a consideration of the experimental study of Amblystoma, it is obvious that by removing the nasal placodes of both sides previous to the formation of nerves, it is possible to obtain larvae which have never possessed functional olfactory organs. Thus is afforded excellent material for a comparative study of the reactions of noseless larvae and normal larvae to olfactory stimuli without the introduction of secondary factors due to shock or discomfort as a result of the operations.

Parker ('10) was the first to show conclusively that aquatic animals react positively to the olfactory stimulus of food. By suspending two bags in an aquarium, the one containing cheese cloth, the other bits of worm, he was able to detect distinct positive reactions of Ameiurus to the bag containing the food material. When on the other hand, the olfactory nerves were severed, no positive reaction occurred. Since then Copeland ('12) has confirmed Parker's results with other fish.

Reese ('12) was the first to attack the problem in Amphibia. Working with Diemyctylus, he showed that the adults would follow and snap at moving bits, whether food or not. They would also make characteristic snapping movements when beef juice was squirted over the external nares. No attempt was made to control the sense of sight. He tried to control the sense of taste, however, by introducing a bit of cotton soaked in cocaine into the mouth. The results were conflicting and inconclusive. Animals in which the olfactory nerves were cut failed to respond to stimulation.

Copeland ('13) repeated the experiments of Reese, using more exact methods. Control of the visual sense was accomplished by stimulating the olfactory epithelium from a motionless source. By dividing the total reaction into two periods, during the first of which an approach was made to the source of the stimulus, and during the second, the object was snapped at or taken into the mouth, he came to the conclusion that the approaching reaction was due entirely to the sense of sight, the seizing alone to the sense of smell.

The object of the following experiments was to test this problem in Amblystoma, comparing the reactions of the normal larvae to food with those of the noseless.

There are in general three groups of sensory organs which conceivably may receive stimuli from the source of olfactory stimulus. These are, in the order of relative importance, the eyes, the taste buds and the lateral line and general cutaneous systems.

Stimulation of the latter systems can be eliminated by using a motionless source, since such stimulation is brought about by currents in the water. Such a source would at the same time give relatively little stimulus to the visual sense.

Control of the stimuli of the taste buds is hardly practicable experimentally without rather serious operations on adult forms. Fortunately it is known that the sense of taste is operative over relatively limited areas and for concentrated fluids only, and hence the danger of stimulation may be minimized by keeping the source of the stimulus at a distance from the mouth (Herrick '08) and Sherrington ('06).

The most important sense, then that must be controlled, is the visual. This may be accomplished in two ways, (1) as stated above by keeping the source of the stimulus motionless and (2) by removing the optic vesicles at an early stage.

In the study of the reactions of Amblystoma to olfactory stimuli, more particularly to the stimulus of food, three sources of stimuli were used, two of which were olfactory and the third purely optic. Pieces of freshly killed Amblystoma larvae and live entomostraca were used to stimulate the olfactory centers; grains of sand, which possess no powers of olfactory stimulus, for the optic centers.

It was necessary to test as exactly as possible the visual reactions of the larvae since, as is evident to the most casual observer, under ordinary conditions, that sense is the most active in the capture of food. Under normal conditions where the food supply is abundant, individual larvae rarely move about, but remain motionless until a small crustacean comes within striking distance. The reactions of larvae under such conditions are quite characteristic. Resting motionless on the bottom of the aquarium, with body held above the debris and head elevated, it will, with a sudden contraction of the trunk muscles followed by a quick forward lurch, snap and engulf some particular crustacean whose movements carry it within striking distance.

When on the other hand, the supply of moving food is reduced, the larvae will forage the aquarium for food. Under these conditions the reactions are quite as characteristic as in the well stocked aquarium. The young Amblystomas crawl slowly around the aquarium, nosing here and there. The attitude is strikingly like that of a dog following a scent, and suggests that now in the absence of food that is moving, the sense of smell is actively used. Support is given to this suggestion by the fact that the larvae may often be seen to snap up some bit of the debris.

In the experimental study of this problem, sixteen normal and twenty-four operated larvae were used. Sixteen of the latter were noseless and eight were eyeless. These last were kindly loaned by Dr. Henry Laurens who performed the operations. As soon as the operated individuals as well as the normal ones isolated at the time of the operation began to feed regularly, experimentation was begun. At this time the larvae are about 1 cm. in length. The yolk has completely disappeared, the gills are plume-like arching forward over the head, the forelimbs are tridigitate, the rudiments of the fourth digit just beginning to appear, and the hind limbs are just noticeable as a slight elevation on either side of the cloaca. Experimentation with these young larvae was difficult because of the very great activity which they exhibited whenever there was any disturbance in the water. For that reason the following report deals with somewhat older larvae, the age varying from one and a half to six months.

The first problem was to determine the relative importance in the obtaining of food of the visual and olfactory sense. For this purpose grains of sand were used, dropped from a capillary pipette so as to fall within striking distance of the larva. A single individual was tested at a time. Four noseless and four normal larvae were subjected to these tests. The first were made when the larvae were five months old, dating from the operation. Another set of exactly similar tests were performed one month later.

The forty tests on the four normal larvae resulted in twenty reactions in which the sand grain was snapped at and engulfed, such reactions being designated as positive, and twenty in which no attention at all was paid to the sand. The percentage of positive reactions then, was fifty. Fifty-four tests on four noseless larvae resulted in thirty-nine positive reactions, a percentage of seventy-two. The same eight larvae tested one month later gave for the normal larvae eleven positive reactions out of forty tests, or 28 per cent and for the operated twenty-two out of forty-four, or 50 per cent (table 1).

TABLE 1

Reactions to moving sand grains

CONDITION OF LARVAE	REACTION	NO REACTION	PER CENT POSITIVE
Normal, 5 months	20	20	50
Normal, 6 months	11	29	27.5
Noseless, 5 months	39	15	71.7
Noseless, 6 months	22	22	50

It is quite evident then, that larvae, whether normal or operated, will snap at almost any object which in any way simulates the movements of the food, no matter whether it stimulates the olfactory sense or merely the visual. An interesting fact was noted in connection with the reaction of the noseless and normal larvae to the sand grains. As will be seen in table 1, there is a marked diminution in the total number of positive reactions of the older larvae as compared with the younger. While the number of reactions is far from sufficient to serve as a basis for any definite conclusion, the difference in behavior suggests that the larvae gradually adapt themselves to the new situation.

The second problem was to determine experimentally whether the olfactory sense played any part at all in the quest for food. Bits of beef were placed in the bottom of aquaria containing normal and operated individuals. The normal specimens would soon nose out the beef and engulf it; the noseless ones never. Exact records were not kept of these tests because the larvae did not thrive on the beef. They would often gorge themselves, much to their own detriment.

Since entomostraca make up the bulk of their natural food, these were used in a series of tests to determine the reaction to olfactory stimulus. By careful handling with a capillary pipette, it was possible to deposit individual Daphnids two or three millimeters from the head of the larvae, so that they would remain motionless on the withdrawal of the pipette. Ninety-five tests were performed on eight normal larvae; eighty-seven of these resulted in positive reactions, a percentage of ninety-two. One hundred and nineteen such tests performed on eight noseless larvae resulted in not a single positive reaction, the individuals would invariably swim off without paying any attention to the Daphnid, or after a time the entomostracan would move away. The above experiments were performed on larvae varying from one and a half to four months old, dating from the time of operation, and indicate that these larvae do make use of the sense of smell in obtaining their food. This was further corroborated by the following tests on the eyeless forms (table 2).

TABLE 2

Reactions to motionless entomostraca

CONDITION OF LARVAE	REACTION	NO REACTION	PER CENT POSITIVE
Normal	87	8	91.6
Noseless	0	119	0.0
Eyeless	119	10	91.8

The eight eyeless larvae were subjected to the same tests. Out of one hundred and twenty-nine, one hundred and nineteen were positive, giving a percentage of ninety-two, a very close correspondence with the normal. Here the olfactory sense was the only one held in common by the normal and eyeless larvae that could receive stimulation.

A curious phenomenon, to be investigated later, was observed in connection with the eyeless individuals. They were very much more sensitive to currents in the water than the normal larvae. A pipette waved in the vicinity of the tail of the eyeless larvae would cause it to turn sharply around and snap at the pipette. No such extreme sensitiveness was observed in the normal larvae. The latter would snap at a pipette but only when waved with the characteristic jerky movement of its normal food. It is suggested that this peculiar action of the eyeless larvae is due to a compensatory hypersensitiveness of the lateral line and general cutaneous systems.

In the spring of 1914, one year after the above experiments were performed, a number of larvae, probably A. opacum, were brought into the laboratory from the field. These were about 30 mm. long—evidently developed from eggs laid the previous season. Five of these were isolated and their feeding reactions tested with small bits of freshly killed Amblystoma larva. Sixty-three tests were made, fifty-two resulting in positive reactions, a percentage of eighty-three. The food was deposited by means of a capillary pipette some 5 to 6 mm. from the head of the larva. Copeland has pointed out that the reaction of Diemyctylus to food is a complex one involving an approaching reaction as a result of a visual stimulus and a seizing reaction due to the olfactory stimulus. In the present experiments on Amblystoma, it was often evident that the placing of the food attracted the attention of the larva. But the stimulus thus imparted was seldom followed by approach to the source. When it was, the test was excluded from the tabulation. If the larva became quiescent after the visual reaction, it would react positively in the majority of cases to the olfactory stimulus by snapping at the food, as indicated above.

In addition to the above tests, bits of larvae were placed in the aquarium as far from the individual under observation as possible, so that stimulation of the optic sense could be practically eliminated. In all of the cases, there was no immediate reaction, though the food was eventually discovered and eaten.

An interesting observation deserves mention in connection with these experiments. Powers in 1907, in studying variations in A. triginum, found that certain of the individuals collected, possessed a remarkable width of head with a correlated expanse of jaw and unusual development of teeth. After watching these individuals, he concluded that the differences "were due to the cannibalistic" habits of the larvae. They would quickly devour young larvae and snap off projecting parts of their fellows. He found only a few such individuals, and concluded the variation was rare.

The larvae in the laboratory at New Haven are probably of the species punctatum, though none have as yet been raised through metamorphosis. But all of these, whether reared from eggs in the laboratory or brought in already hatched, would readily eat one another, or such parts of limbs, gills and tail as were obtainable. This cannibalism did not, however, appear in the larvae until some time after feeding had commended. When it appeared, there seemed to be no correlation between it and the amount of normal food, entomostraca and plant life, available. Of those in the aquaria in the laboratory there were a few that could not be induced, even after starving, to touch parts of other larvae. The older larvae brought in were almost all maimed in some part or other. On being isolated, the missing parts were soon regenerated, producing perfect specimens. It is obvious, then, that cannabalism is not as rare in A. puntatum as Powers believed it to be in A. triginum.

MORPHOLOGICAL EFFECTS OF OPERATION

I. The skull

Normal development. The normal development of the chondrocranium of the Amphibia has been fully described. Platt ('97) has considered carefully the origin and early history of cartilage in the head of Necturns. The later development has been studied Winslow in 1898, by Wilder in 1903, and Gaupp, 1905. The normal development of the nasal cartilages, particularly in Amblystoma has been fully described by Terry in 1906.

In order to grasp fully the effect of the absence of nasal epithelium on the formation of cartilage in the head region, a brief account will be given at this point of the normal development as outlined by Terry and confirmed from the controls of the present series of experiments. Occasion, has been found, however, to change the nomenclature used by Terry, preference being given to the terminology of Gaupp ('05).

The first evidence of cartilage in the head region is the appearance of two centers of chondrification on the latero-ventral aspects of the diencephalon. From these centers the mesenchyme becomes chondrified in an antero-posterior direction forming two rods of cartilage, the trabeculae. The posterior growth does not concern us here and further consideration of it will be omitted.

Chondrification proceeds anteriorly until a cartilaginous rod is formed reaching to the anterior face of the olfactory sac. At the same time the mesenchyme interposed between the eye and the brain is converted into a flat plate of cartilage, the crista trabeculae, by chondrification, which, starting from the tabecula, proceeds dorsally. In the early stages this plate does not extend anteriorly beyond the caudal end of the olfactory sac. With the formation of these cartilages, there appear two processes, one a lateral process—the antorbital, and the other a median process. The antorbital appearing first as a more or less straight rod projecting outward, eventually becomes hooked anteriorly about the posterior nares.

The median process projects medially from the trabecula, just in front of the anterior end of the telencephalon. It eventually meets and fuses with its fellow from the opposite side. This solid bar, whose caudal margin is later thinned out into the thin floor of the anterior part of the cranium, becomes the ethmoidal or anterior trabecular plate.

With the advancing chondrification of the trabeculae is correlated the formation of a cartilaginous plate underlying the nasal sac, supporting it and Jacobson's organ. This solum nasi extends under only the anterior two thirds of the olfactory organ.

During all these earlier stages of development the nasal placode is closely applied to the latero-ventral surface of the telencephalon. As growth proceeds, the sac begins to push anteriorly and at the same time laterally, leaving thus a clearly marked olfactory nerve. In the mesenchyme which lies above this early olfactory nerve there appears as a separate chondrification a small rod of cartilage—the ethmoid column. This grows in an antero-posterior direction, becoming fused posteriorly with the crista trabeculae. Anteriorly, it grows toward the trabecula sending a short process to meet it before attaining to its maximum length. In front of this process is formed the narrow slit, the medial incisure through which passes a branch of the nasalis internus. Subsequently this slit is reduced to the foramen apicale. The growth of the ethmoid column posteriorly to unite with the crista and anteriorly to join the trabecula forms a bridge over the olfactory nerve, which constitutes the roof of the fenestra olfactoria (fig. 8 *fo*). At the same time there appears the medial nasal process. As is shown in figure 8 *mp.*, this is a thin plate of cartilage that lines the medial and anterior surface of the nasal capsule, between the sac and the premaxilla.

Correlated with the posterior growth of the ethmoid column is a lateral chondrification of the mesenchyme overlying the medial-dorsal aspect of the nasal sac. In this manner is formed a roughly rectangular plate of cartilage whose lower surface is concave to fit the dorsal aspect of the sac, and whose posterior edge marks in a general way the anterior boundary of the orbit. This is the tectum nasi (fig. 8 *tc.*).

The cartilages formed in the above manner almost completely surround the olfactory sac, only the region about the external and the internal nares is left uncovered.

Development after operation. The first visible effect of the removal of the nasal sac is a sinking in of the skin in the nasal region. In the case of the bilateral operations this is so marked as to give a veritable 'pug dog' effect, which increases with age. Figure 10, a drawing of a section through the head of a larva with a unilateral operation, shows how marked this caving in is.

A study of sections of these unilaterally operated larvae shows at once that most of the capsular structures that arise in connection with the olfactory sac are absent. The ethmoid column, the tectum nasi, and the medial nasal process are all absent. There is, however, a slight indication of a solum nasi. The anterior trabecular plate is apparently regularly formed, though thicker than is normally the case on the operated side. The antorbital process is also formed, though, as a glance at figure 6 will show, it is atypical in form and position.

The trabecula of the operated side is very much thickened (fig. 11). In addition the ascending process of the premaxilla has become connected laterally with the maxilla by a thin plate of bone, bridging over the region that would have been occupied by the external nares, figures 5 (*bp*) and 10. The formation of this unusual bony connection has apparently drawn the maxilla in, so that it no longer possesses the normal curvature (fig. 6). The line of the lower jaw follows that of the upper perfectly. There is in the lower jaw a compensating curvature to that of the upper, so that where the upper jaw curves in as a result of the operation, the lower jaw follows. This brings the teeth into apposition at all times. The maxillary teeth are unaffected by the operation. They are normal as to both size and number on the operated side.

The process by which the above changes in structure are formed is relatively simple. In the operated larvae, the trabecula of the operated side appears simultaneously with that of the unoperated side and for some little time no difference can be observed in the development of the two sides.

Soon after the larva has begun to feed, it becomes evident that the trabecula of the operated side begins to lag behind its fellow in the advancing chondrification. At the same time that this observable retardation in longitudinal growth occurs there is a corresponding increase in the cross section of the trabecula. The antorbital process appears at this time though it is somewhat farther forward that its normal fellow. As development proceeds it becomes more and more atypical, finally appearing as an irregular projection from the trabecula at the level of the posterior terminus of the maxilla.

Coincident with the growth of the cartilages in the region of the posterior nares, there appears laterally the plate-like chondrifications which eventually form the tectum nasi and the solum nasi. The appearance of these on the unoperated side presages a marked thickening of the trabecula of the operated side. The end result of this is shown in figure 11 (*t*). Eventually the discrepancy in the length of the trabecula mentioned above is compensated for, both reaching forward to the premaxilla.

It is evident then that correlated with the absence of the nasal sac, there is an almost complete absence of the normal structures. The process of this reduction may be largely explained on a mechanical basis.

In the early development of the head region, the brain, together with the anlagen of eye and nose fill practically the whole of the anterior part of the head. There are, however, certain inequalities in the contour of the organs which are filled with mesenchyme, thus giving the smooth oval of the head. For example, a section through the head of a young larva at the level of the eyes, shows roughly three circles. the two optic vesicles and the diencephalon enclosed in a fourth, the skin. Between these organs above and below lie two triangular depressions filled with mesenchyme. From the two ventral masses arise the trabeculae. As the eyes become separated from the brain the mesenchyme filling the depression above shifts, gradually becoming chondrified in the process and forming the crista. Exactly the same procedure may be imagined to occur at the level of the olfactory placodes. Here, however, owing to the greater extent of the olfactory nerve, no crista is formed, the mesenchyme of the upper depressions becoming secondarily chondrified to form the ethmoid column.

If now the placode of one side is removed all of the mesenchyme filling the depression simply and naturally joins the trabecular mesenchyme. Hence, no ethmoid column is formed, the mesenchyme from which it is normally developed becoming united with that of the trabecula, greatly adding to its mass.

Figures 5 and 8 from drawings of the model will show that the crista too, of the operated side is not so high as its fellow. It is quite evident that some of the mesenchyme tissue destined to form the cartilage of the crista has, owing to the absence of the placode and olfactory nerve which usually serves as a dam to keep it in position, simply slid forward, reducing the crista forming mesenchyme and adding again to the trabeculae.

The presence of the antoribital process on the operated side indicates that the removal of the nasal sac has not eliminated some factor that determines its formation, though the irregularity in shape and position shows the lack of some formative influence.

The presence of a rudimentary plate of cartilage in the general position of the solum nasi seems to point to a formative influence still present in that region. A careful study of the early stages shows that this plate does not develop until the bony connection is established between the ascending process of the premaxilla and the maxilla. This latter plate in all probability molds into shape laterally a portion of the mesenchyme which later chondrifies to form the rudimentary solum nasi.

The structure of the normal nasal cartilages shows quite clearly that they are an almost perfect 'cast' of the underlying nervous tissue. The removal of the 'core,' the nasal sac, of the capsular cartilages causes a collapse of the whole structure with the subsequent settling of the cartilage forming material to the lowest point.

It may be concluded from these experiments that there exists in the head a certain amount of mesenchyme tissue destined to form cartilage. The general shape of these cartilaginous structures is determined by the form of the underlying organs. The removal of any such underlying parts results not in any reduction in the mass of cartilage formed, but in the rearrangement of the cartilage forming tissue to meet the change in the form of the 'core' about which it is formed.

II. Development of naso-lacrymal duct

In addition to the modifications of the capsular structures, it was noted that the naso-lacrymal duct present in the 33 mm. larva, on the unoperated side was entirely absent on the operated.

At present the most widely held theory of the formation of the naso-lacrymal duct is that it is a solid cord of cells growing centripitally toward the nasal epithelium from the point of origin in the ectoderm on the dorso-anterior aspect of the optic anlage. This solid cord becomes secondarily canalized.

Figures 10 and 11 (*nl*) show respectively the junction of the cord with the nasal epithelium and the surface ectoderm. It is rather thicker at the point of union with the nasal sac than where it meets the ectoderm and seems to run more gradually into the sac than it does into the ectoderm. There is also a marked continuation of the cavity of the sac into the cord indicating that the canalization occurs from this end. Born in 1876 worked out the development of this duct. He found that it first appears as a solid cord of cells reaching from the surface ectoderm to the nasal sac and that it is derived from the former. The work of Schaeffer in 1912 on man has shown that the duct is at first a solid cord derived from the inner layer of the epidermis.

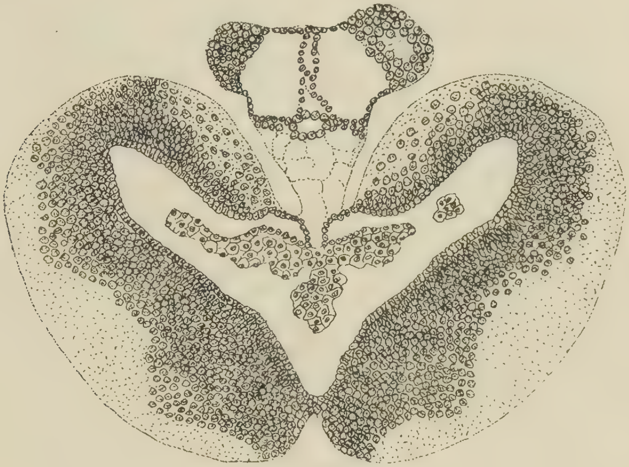
The suggestion is made here that it would be possible by very careful operating to remove the nasal analge without disturbing the duct rudiment, and vice-versa. The material at hand rather indicates that the duct is derived not from the surface epithelium but from the cells of the nasal sac. The above operations would make it possible to arrive at a definite conclusion experimentally so far as Amblystoma is concerned as to the exact origin of the duct.

III. The Brain

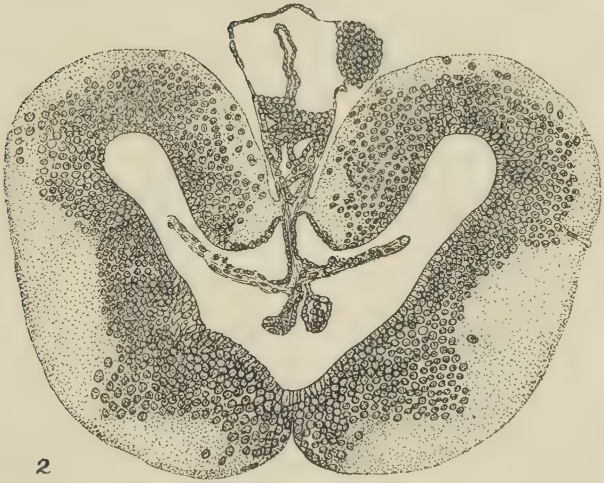
Owing to the difficulties encountered in keeping noseless larvae alive up to metamorphosis, the report on the effect of the absence of the olfactory organ on the fiber tracts of the forebrain must be postponed until later. The effect on the gross morphology can, however, be given in its entirety at this time. For the sake of clarity the description of the brain of the six months old larvae will be given first, since, as will be shown, the effect of the removal is not immediate.

Figure 7 will make evident at once the effect of the absence of the nasal sac on the gross morphology of the telencephalon. The hemisphere of the operated side is markedly smaller than that of the unoperated. This reduction in size, however, is related largely to the anterior portion of the telencephalon. In other words, the olfactory bulb as such is greatly reduced in size—a reduction which is apparent also for some distance caudally. In the region of the primitive hippocampus, on the other hand, the two hemispheres are equal, barring slight unevenness in the sections (text figures 1, 2 and 3).

A study of the cross sections of brains of operated larvae show quite as strikingly the above facts. Figure 12 through the olfactory bulb of the unoperated side shows no sign of the hemisphere of the operated side. Figure 14 from a section some 170 μ caudad shows the right is greatly reduced in size. A section still further caudad at the level of the interventricular foramen shows no distinguishable differences in the two hemispheres other than that in the size of the ventricles. A comparison of text figures 1, 2 and 3 will make evident that there is a progressive enlargement of the ventricles from the normal through the brains of the unilaterally operated larvae to the bilaterally operated larvae. It is not possible at this time to explain the significance of this difference if any exists.

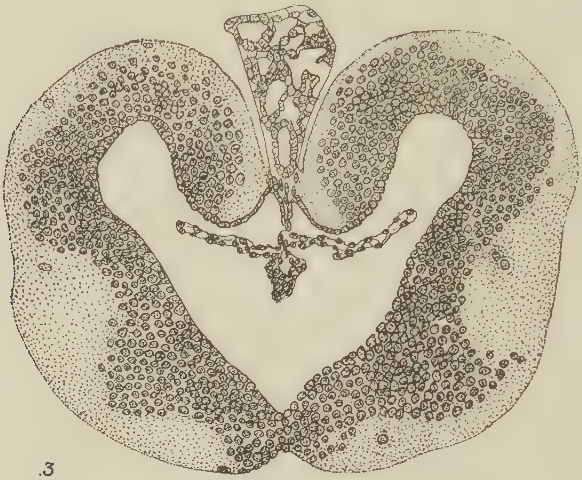


1



2

Sections through the brain, at the level of the hypothalamus, show absolutely no asymmetry in any of the operated forms (text fig. 4).



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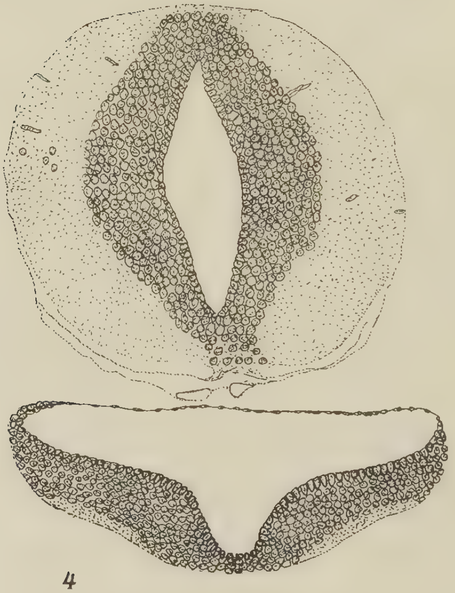

4

It becomes apparent then that the absence of the nasal sac has here resulted in a reduction in the size of the telencephalon of the operated side, the reduction being limited to the anterior portion of the hemisphere.

It is perhaps best at this point to mention one of the results of the operations on the histogenesis of the brain that has been brought out by the technique employed in studying the gross morphology. It is a well known fact that the growth of the olfactory nerve is centripetal,—from the olfactory placode to the telencephalon. The terminations of the ingrowing nerve fibers are known as glomeruli. As would be expected, the absence of the olfactory nerve results in the complete disappearance of the glomeruli. So that there are present in the telencephalon of the operated side only secondary and tertiary tract fibers, the primary fibers being entirely absent.

The material so far studied has as yet shown no discernible effects on these secondary and tertiary tracts. Gudden's atrophy is necessarily a slow process, and it is evident from the material at hand that if they are to show this type of atrophy the operated larvae must be kept at least to metamorphosis. It is probably, too, that the atrophy would continue with age so that metamorphosed larvae would show the most marked differences.

A study of entire brains dissected out of normal and operated larvae, together with a careful inspection of transections of the brains of parallel stages shows that the reduction in size is not immediate. The first four weeks of development after the operation show no differences in the size of the telencephalon. That is, the growth of the two hemispheres during the first part of larval life, three weeks of which have been dependent on yolk and the fourth on the food in the environment, has not been in the least affected by the absence of the nasal sac. From the fourth week on, however, the hemisphere of the unoperated side begins to grow more rapidly than its fellow of the operated side, finally outdistancing it to the extent shown in the drawing of the model.

The fact that the difference in growth did not occur until active feeding had commenced suggested at once that here was a correlation between structure and function (page 34). The physiological experiments showed that larvae over four weeks old could detect food by the sense of smell. Correlated, then, with the beginning of functional activity of the nasal epithelium, there exists in operated forms the beginning of a difference in the growth rates of the two hemispheres.

The above facts suggest that there is inherent in the tissue of the central nervous system a certain potential for development and differentiation. This potential carries the growth up to a certain point at which all the parts of the brain are present. The further growth then becomes dependent on the functional activity of the parts.

The conclusion stated above can not be considered final. A more extensive study with special methods, of the fiber tracts involved must be engaged in before any final judgment can be reached. At present such a study is under way. It is planned to carry the operated larvae through metamorphosis as it is evident the effects become more pronounced with age.

The writer takes this opportunity to express his appreciation of the criticism and suggestions made by Dr. Harrison during this investigation.

SUMMARY

The physiological tests of the reactions to food of normal and noseless larva show:—

1) That normal larvae will react positively to sand grains that move in the water.

2) That normal larvae will react positively to food that is not moving.

3) That noseless larvae will react positively to sand grains but not to motionless food.

4) That eyeless larvae will react positively to food that they cannot see and will not react to moving sand grains.

The effect of the absence of the nasal sac on the form of the skull is shown in the complete collapse of the cartilages that normally surround the sac, this collapse being due to the absence of the support given by the nasal placode to the mesenchymal tissue in which the cartilage is formed.

The effect of the absence of the nasal sac on the brain is a reduction in size of the telencephalon.

The removal of the nasal epithelium deprives the developing forebrain of a stimulus necessary for its complete development. This is evidenced by the fact that the forebrain of the six months old larva from which one placode had been removed, showed considerable differences in the size of the two hemispheres, the operated side being the smaller. This difference however is found only after the nasal sac of the unoperated side has become functional. This fact indicates that there exists in the central nervous system as, indeed, in other organs a "potential for differentiation" which carries the development of the parts to a point at which all parts are present although not in their final form or size, the further growth being dependent on the functional activity of the end organs of the brain.

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PLATE 1

EXPLANATION OF FIGURES

5 Three quarter view of model of skull of 33 mm. larva from the right side.

6 Dorsal view of above model. Drawn to smaller scale than figures 5, 7, 8.

7 Dorsal view of model of brain of unilaterally operated 33 mm. larva.

8 Three quarter view of model of skull of unilaterally operated 33 mm. larva from left side.

ABBREVIATIONS

ao, antorbital process
ap, anterior trabecular plate
apm, ascending process of premaxilla
bp, bony plate connecting premaxilla and maxilla
ct, crista trabeculae
ec, ethmoid column
fo, fenestra olfactorius
m, maxilla
mp, medial nasal process
pm, premaxilla
sn, solum nasi
t, trabecula
tc, tectum nasi

Fig. 5

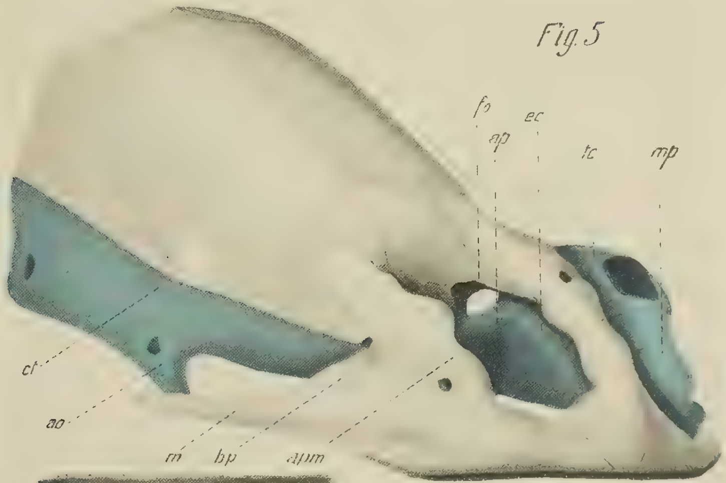


Fig 6

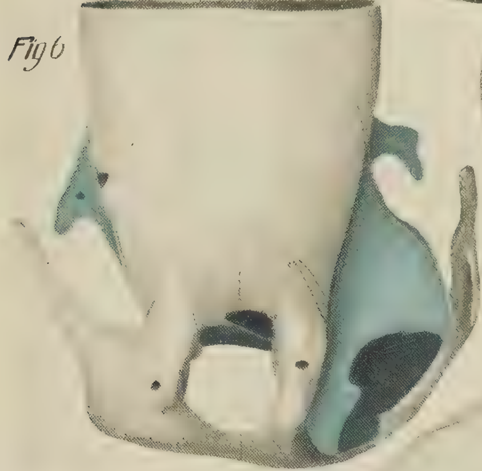
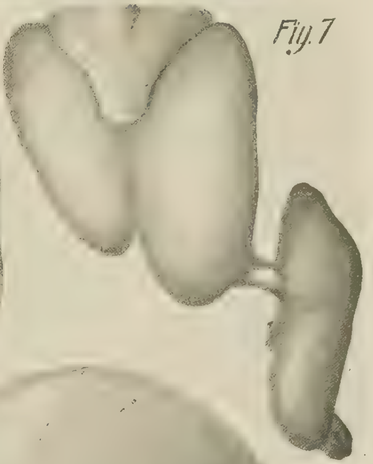


Fig. 7



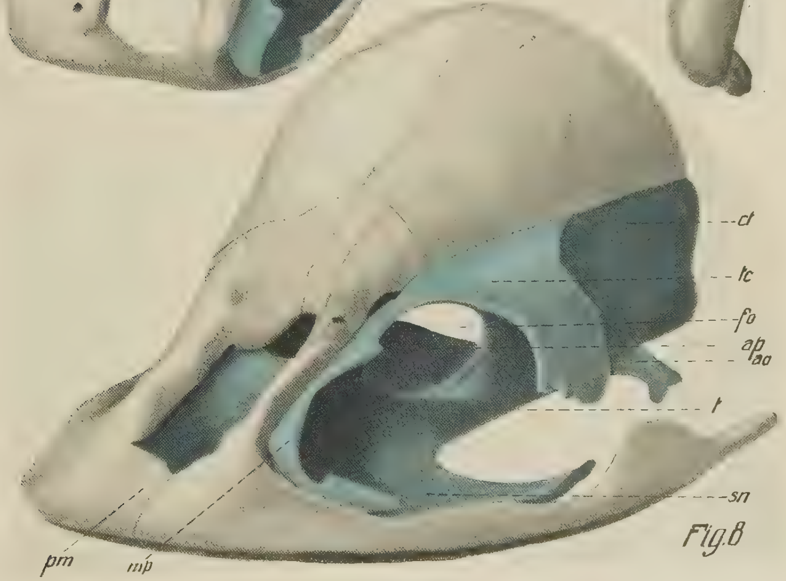


Fig. 8

PLATE 2

EXPLANATION OF FIGURES

9 Cross section of head of a unilaterally operated larva showing reduction in the height of crista (ct) and in the size of the right telencephalon. × 100.

10 Cross section 690 microns anterior to figure 9 showing bony plate connecting maxilla and premaxilla, the caudal end of the naso-lacrymal duct, the tectum and solum nasi and the ethmoid column. × 100.

11 Cross section 410 microns anterior to figure 9 showing the left telencephalon with its glomeruli and no sign of the right, the distal end of the naso-lacrymal duct and on the operated side the much thickened trabecula. × 100.

ABBREVIATIONS

ap, anterior trabecular plate
apm, ascending process of premaxilla
bp, bony plate connecting premaxilla and maxilla
ct, crista trabeculae
ec, ethmoid column
m, maxilla
nl, naso-lacrymal duct
ns, nasal sac
sn, solum nasi
t, trabecula
tc, tectum nasi
tel, telencephalon

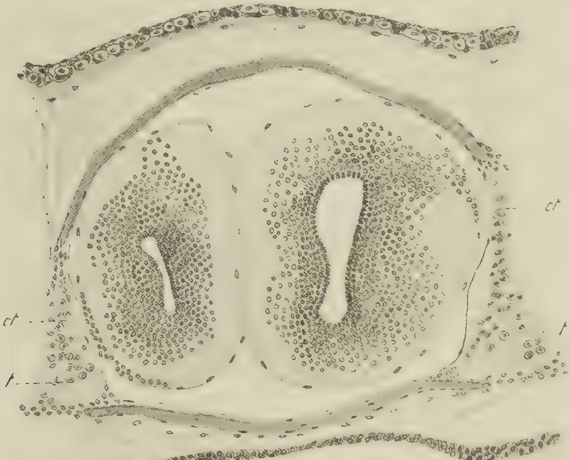


Fig.9

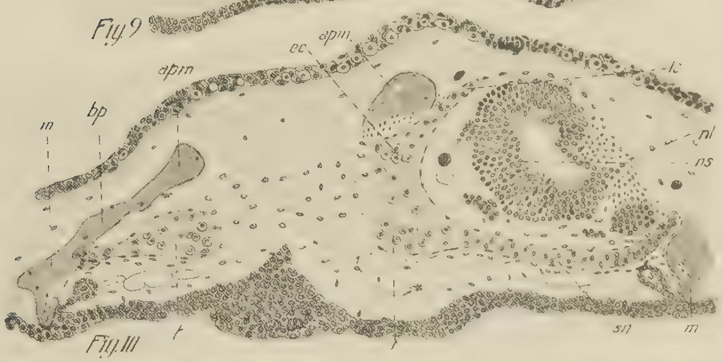


Fig.10

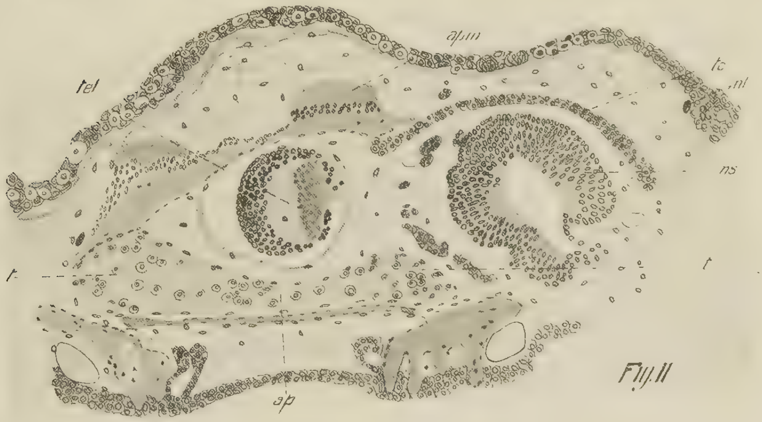


Fig.11

PLATE 3

EXPLANATION OF FIGURES

12 Cross section of the forebrain of a unilaterally operated larva through the olfactory bulb showing the glomeruli and the absence of the olfactory bulb on the operated side. × 160.

13 Cross section of the forebrain of a normal larva at same level as figure 12 × 160.

14 Cross section of the same forebrain that is figured in figure 12, 17 microns caudally showing the first signs of the right telencelphalon. × 160.

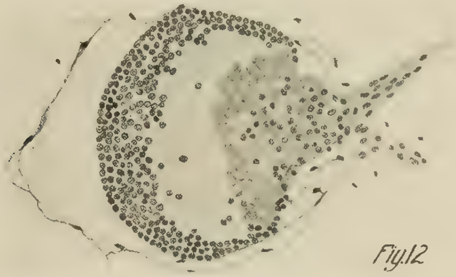


Fig.12

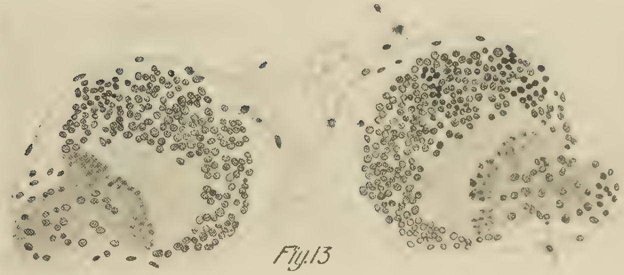


Fig.13

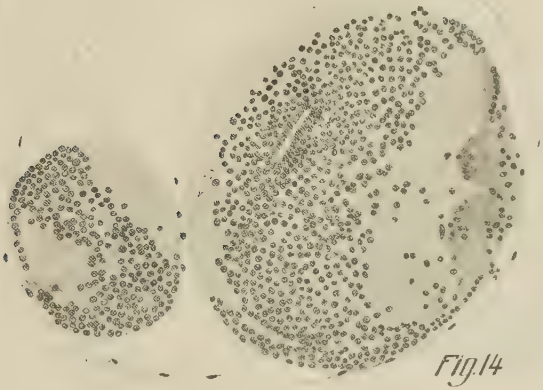


Fig.14

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February, 1916

THE EFFECT OF LIGHT ON THE RETINA OF THE TORTOISE AND THE LIZARD

S. R. DETWILER

From the Osborn Zoological Laboratory, Yale University

ELEVEN FIGURES

INTRODUCTION

Since the discovery of the migration of pigment by Boll ('77) and by Kühne ('77) and of the contraction of the cones by van Genderen Stort ('87,) (see Engelmann, '85) in the retina of the frog, this subject has been carefully investigated by many authors in many animals. Garten ('07) has brought together the main and important results of this work on the changes induced in the retina by light. From this we see that light produces a variety of effects on the form and staining reactions of the different parts of the retina. Of these effects three are particularly interesting to us. 1) Migration of the pigment in the epithelial cells of the retina. 2) Changes in form and position of the visual cells. 3) Changes in form, position and ability to stain of the ganglion cells and of the nuclei of the inner and outer granular layers.

As far as the reptiles are concerned these questions seem far from settled, and therefore worthy of further investigation. In the first place concerning the migration of pigment, Angelucci ('78, p. 372) was not able to say from the results of a few experiments on the turtle, Testudo graeca, and on lizards (L. agilis, L. muralis and L. viridis), which have no rods, whether pigment migration took place or not. If it does, he remarks, it is much less marked than in the amphibian eye. Boll ('81, pp. 20 and 21) in an incompleted work, also considered this matter and in a theoretical consideration of the physiological properties of the pigment epithelium, in which he regards the migration of the pigment to be bound up with the using up and regeneration of visual red in the rods, he concludes that pigment migration should not take place in a rodless retina, such as the lizards have. Boll, however, cannot say whether it does or not.

Angelucci ('94) (see Garten, p. 68), however, found that in Testudo marina the pigment does migrate, though less strongly than in the frog. And Chiarini ('06) was also able to clearly demonstrate pigment migration in the retina of the lizard (L. agilis). He figures, rather diagrammatically, side by side a dark and light retina, and, although he gives no measurements of the extent of the migration, it is clear that the pigment in the illuminated eye is nearer the external limiting membrane than it is in the dark eye, covering the paraboloids and drawing away from the bases of the pigment cells so that their nuclei are entirely uncovered.

Garten ('07, p. 68), however, was unable to obtain preparations of the retina of Emys, Chameleon or of Lacerta which showed constant differences in the position of the pigment according as to whether the animal had been kept in darkness or in bright light. His results will be referred to again. And finally Hess, ('10, p. 281), was no more successful than Garten with Emys europaea, the position of the pigment in eyes of individuals that had been kept for 22 hours in darkness, 2 hours in sunlight and several hours in light of weaker intensity being in all not markedly different, the outer segments being always covered by a mantle of pigment.

Concerning the contraction of the cones in light, Engelmann ('85, p. 500) found that in the eye of the snake Tropidonotus natrix, which contains no rods in the retina, the cones contracted but little. Also that in Testudo graeca it is doubtful whether any contraction takes place. Angelucci ('94) (see Garten '07, p. 25) however, claims that in Testudo marina contraction of the cones does take place, though less in extent than in the frog. Chiarini ('06) also reports that in the eye of L. agilis the cones shorten when the eye is brightly illuminated, but only slightly, for the cones measure in dark eyes 25–35μ, in light eyes

23–30μ. Finally Garten ('07, p. 25) found also a very slight contraction (not more than 1.1μ) in the eye of Chameleon.

From this brief review of the few papers on the subject concerning reptiles we see that pigment migration and cone contraction are very slight if they occur at all. No work of this nature has been carried out on American species, and since it is desired to carry out a series of further experiments on tortoises and lizards with particular reference to vision, it was thought that something should be known concerning the reactions of the various parts of their retinae to light. Three species of tortoises and one of lizards were used in the present investigation, viz., Chelopus guttatus, Chelopus insculptus, Chrysemys picta, and the common southern fence lizard, Sceloporus undulatus. Most of the work was carried out on Chrysemys and Sceloporus.

This investigation was taken up at the suggestion of Dr. Henry Laurens. It gives me pleasure to express here my thanks to Dr. Laurens for the assistance that he has given me during its completion.

METHODS

The methods of exposing the animals to light and to darkness were as follows: Two active animals were selected and placed in darkness for 24 hours. At the end of that time one of them was taken from the dark room and placed in direct sunlight for at least 6 hours after which it was killed. The other animal was either killed after it had been in darkness for 24 hours or after it had remained there at least 6 hours more. The eyes were removed as quickly as possible after the animals had been killed by decapitation—the dark eyes under red light, the light eyes in sunlight—and immediately dropped into the fixing fluid. The time consumed between decapitation and fixation was 5 minutes or less.

The fixation and subsequent procedure which gave the best results was the following: Fixation in Kleinenberg's strong picrosulphuric for 4 to 5 hours, followed by 70 per cent alcohol, which was frequently changed and in which the eyes were allowed to remain for several days. Further dehydration, consuming at least 2 days, the lens being removed after the eyes had been in 95 per cent alcohol for several hours. For infiltration the chloroform parafin method was employed, paraffin melting at 52° C. being used for imbedding. Sections were cut 8 to 10μ thick, stained in Ehrlich's haematoxylin, followed by eosin. In a few cases, in an attempt to secure a quicker and perhaps more perfect fixation, the lens was removed before the eyes were dropped into the fixing fluid. It was found, however, that this method caused shrinkage and folding of the retina to such an extent, at the same time producing no better fixation, that it had to be given up.

ANATOMICAL

Before we proceed to consider the results of the comparison of light and dark eyes it will be best to give a brief account of the anatomical relations of the species with which we are working. Considerably more has been done by previous investigators on the morphology of the reptile retina than on its physiology, and it will be well to review briefly the results of this work.

Schultze ('66 and '67) noted that in Emys europaea, L. viridis, L. agilis and L. muralis there were no rods. From Hulke's ('67, p. 94) incomplete description one might assume that rods were to be found in the retinae of, among others, Testudo graeca, Emys europaea, Chelone midas, Lacerta viridis and Anguis fragilis, though, as Krause pointed out later, these 'rods' are more correctly to be considered as cones without oil drops. Heinemann ('77, p. 423) who examined the retinae of several Mexican species of tortoises concluded that, if the form of the outer segment be taken as the criterion, then rods as well as cones can be distinguished in the retina of Chelonians. That in lizards, however (p. 431) no elements with rod-like outer segments can be distinguished. In the Geckos, however, (p. 434) it is doubtful whether the visual elements are rods or cones.

Angelucci ('78, p. 371) found that rods are entirely lacking in Testudo graeca, L. agilis, L. muralis and in L. viridis. Boll

('81) briefly states (pp. 21 and 35) that there are no rods in the retina of Testudo or of Lacerta, while Cheivitz ('89, p. 143) adds that in Emys europaea and L. viridis there is but one form of visual cell which, from its form, is a cone.

Krause ('93) finds that in Chelonians there are no rods, and that the elements which had been earlier described as rods were nothing more than cones without oil drops. In L. agilis, however ('76 and '93), he describes rods as being present, but scarce, though in certain places they are found thick together. Anguis fragilis and L. viridis have only cones.

According to Angelucci, ('94, see Garten, p. 25) Testudo marina has no rods and Greeff ('00, p. 123) makes the brief statement that the reptilian retina (lizards, snakes and tortoises) has only cones. Chiarini ('06) says that the neuro-epithelium of lizards is formed exclusively of cones of various sizes. Garten ('07, p. 24) points out that in Tropidonotus there are no rods, that in Testudo graeca there are probably none, and that in the Chameleon there are certainly no rods.

Pütter ('09, p. 103), concludes from the mode of centripetal connection (dendritic) of the visual cells of the reptiles that all of them must be cones, although the form of the single elements can be very different, e.g., in Anguis fragilis and in the Gecko there are found cylindrical or rod-like outer segments. But he adds that all Chelonians have conical outer segments.

Hess ('10, p. 281) gives a review of the literature and states, that, in addition to the anatomical features of the retina indicating that there are no rods, the futile endeavors of several investigators to obtain evidences of visual purple in the tortoise retina indicates that there can be no rods present. And finally Franz ('13, p. 52) in a very incomplete and, in some particulars, incorrect review makes the statement that tortoises possess both rods and cones.

From these papers the conclusion may be drawn that cones are by far the principal visual element of the retina of tortoises and of lizards. Further that rods may occur in a few, but that they are scarce. It is to be regretted that good figures of the visual cells are not given in any of the articles reviewed. Moreover except in the work of Heinemann and Krause, American species have not been investigated. Of those mentioned by these authors only one genus is represented, viz., Sceloporus, that has been studied by me, and this is a Mexican species. The need therefore for some account of the retina of the ordinary American species of tortoises and lizards is still urgent, and since the anatomical features of the visual cells have not yet been described, a short account of these will not be out of place.

To begin with the tortoises we find that in the retina of the three species examined there are no rods. The cones are of two sorts, single and double. The single cone is the more numerous type of visual cell, and they are all similar in the possession of an outer and inner segment, in the latter of which is found in all cases an oil drop, an ellipsoid and a paraboloid. In form and size, however, these single cones present individual variations, on the basis of which we may say that there are two kinds, the first of which is considerably broader than the other, but only a little longer. With this increase in size there is found a slightly larger paraboloid and oil drop (fig. 6).

The double cones, of which there is only one kind, are much fewer in number than are the single cones. They are composed of a principal and of an accessory part, there being no twin cones. The principal cone has a very long narrow myoid, a long ellipsoid and an oil drop, there being no paraboloid. The accessory, which is much broader and shorter than the principal cone, has the typical short myoid of a single cone, a paraboloid, a granular ellipsoid but no oil drop (fig. 6).

From preparations of fresh retinae it was found that the colors of the oil drops were those which have been usually described for the tortoise retina, namely red, orange, pale yellow and blue green. The red are the largest and the most numerous.

Krause ('93) describes single and double cones in the retina of Emys europaea, there being two varieties of each. The first kind of single cone is similar to that which we have just described, the second is much broader and has only a coarse granular ellipsoid, but with no oil drop or paraboloid. Concerning the double cones of Emys the first variety, which is extremely numerous, is similar to those just described, differing only in that both the principal and the accessory cones may have oil drops. The principal cone has a plano-convex ellipsoid, while the accessory possesses a plano-concave ellipsoid and a homogeneous paraboloid. In the second variety of double cone the principal cone is similar to the first, but the accessory is similar to the second variety of single cone, that is, thicker, with no oil drop but with an ellipsoid.

Heinemann ('77, p. 423) from his study of the retinae of several Chelonians distinguishes two kinds of cones. 1) those with an oil drop, and 2) those without. Of the first of these the inner segments are much thinner than the others, so that they approximate the form of rods, containing an ellipsoid and a paraboloid. Heinemann subdivides these cones with oil drops into four varieties. a) those with bellied out inner segments and large lens-shaped bodies, b) those which are narrower and with a smaller body, c) those which are pointed on the inside and contain here either a body of appropriate size or none at all, d) cones with strongly bellied out outer portion of the inner segment and with irregularly formed, and always much narrower, inner portion of the same. Seldom there is to be found here a small lens-shaped body, but usually this part is structureless or filled with a finely granular mass. The last two kinds, c) and d), of cones with oil drops unite with the cones without oil drops to form double cones. But in Testudo gray, double cones, both parts of which contain an oil drop are also to be found.

Concerning the lizard retina here again it may be said that there are no rods. The cones, however, in addition to showing several varieties among themselves are different from those of the tortoises examined (compare figs. 6 and 7). We again find in the first place that there are single cones and double cones. The single cones are of two varieties, a) with a very long myoid, no paraboloid, but with an ellipsoid and an oil drop. And b) much thicker than the first, with a shorter myoid and with an ellipsoid, paraboloid and oil drop (fig. 7). Similar to the tortoise retina there is one kind of double cone. The principal cone is similar to the long narrow single cone while the accessory is much thicker, with a large paraboloid, a granular ellipsoid, and no oil drop (fig. 7).

Krause ('93) holds that Lacerta agilis has four kinds of visual elements, one of which is a rod, the other three being cones. The second kind of single cone is distinguished from the first by having a thicker inner segment and lacking an oil drop, although there is present an ellipsoid and a paraboloid. Double cones come principally from a combination of a principal cone with an oil drop and an accessory cone without an oil drop, though he also found double cones, both parts of which contained an oil drop. Heinemann ('77) finds two kinds of cones, those with oil drops and those without. The cones with oil drops show all gradations from voluminous cones with large lens-shaped bodies to very thin ones with very narrow inner segments in which there is no lens-shaped body. These latter unite with cones without oil drops to form double cones.

Greeff ('00, p. 117) figures a single and a double cone for the retina of a lizard. The single cone is quite similar to the narrow type found in Sceloporus (fig. 7), the only difference being that the myoid is comparatively shorter and thicker. The second or broad type of single cone, which is the predominating type of single cone in Sceloporus, he does not figure at all. The double cone is entirely similar to the one found by me.

In passing, it is interesting to note that in the tortoise retina the external nuclear layer consists of two rows of nuclei. Of these only those nuclei in the row immediately internal to the external limiting membrane are cone nuclei, the other row being bipolar nuclei which connect the cone nuclei with those of the inner nuclear layer. The cone nuclei are in general larger and more oval, with the long axis in the same line as that of the cones. In Sceloporus, however and Cnemidophorus (the sand lizard) the external nuclear layer consists of only one row. Chievitz ('89, p. 146) considers the second row, which he found in both Emys and Lacerta, to be the nuclei of supporting cells. Another point of interest is the presence of a fovea and large papilla in the lizard retina, but neither of these in the tortoises, though there is a small area centralis. Heinemann ('77, p. 425) could find no fovea in the retinae of any of the Chelonians which he examined, although in several there was a small papilla. Chievitz ('89, p. 143) describes an area for Emys but no fovea, while in Lacerta viridis (p. 147) he finds a fovea.

EXPERIMENTAL

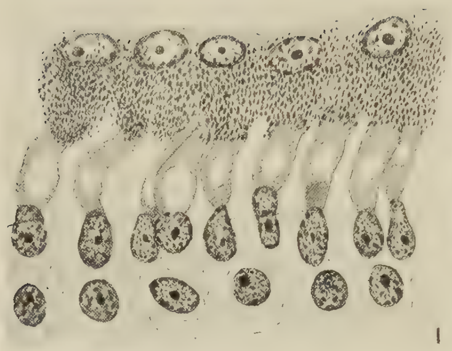
When sections of eyes taken from animals which had been placed in sunlight are compared with sections of eyes from animals kept in darkness it is seen, in the first place, that pigment migration does take place (figs. 1 and 2). In the dark eye (fig. 1) the pigment occupies more of the body of the epithelial cell, so that the nuclei are for the most part covered, and extends forward just beyond the oil drop, which in a few cases can be seen through the pigment. In the light eye (fig. 2) it is seen that the pigment has migrated forward so that the pigment cell nuclei are almost entirely uncovered while the pigment extends further toward the external limiting membrane, in many cases as far as the paraboloid. It was also noted that in the light eye the pigment epithelium adheres more closely than in the dark eye and is not so easily torn away.

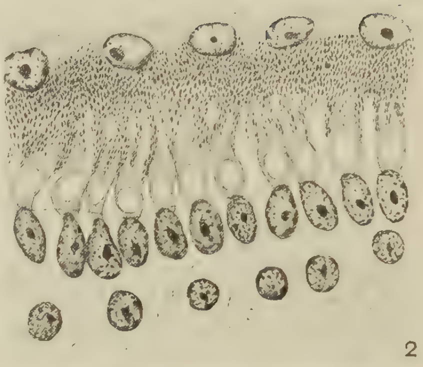
The results of a series of measurements to determine the extent of migration are shown in table 1 (column 3). It will be seen that the average distance from the external limiting membrane to the nearest pigment needle in the dark eye is 8.8μ while that for the light eye is 5.2μ. The difference of 3.6μ represents the extent of the migration.

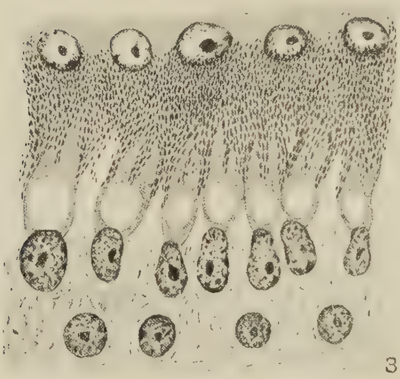
TABLE 1[1]

ANIMAL	DIST. FROM EXT. LIMIT. MEMB. TO CHOROIDAL EDGE OF PIGMENT EPITHELIUM	DIST. FROM EXT. LIM. MEMB. TO OUTER SEG. OF CONE	DIST. FROM EXT. LIM. MEMB. TO NEAREST PIGMENT NEEDLE
Chrysemys			
Dark..........	26.0μ	20.7μ	8.8μ
Light.........	21.0μ	18.4μ	5.2μ

[1] Based on 10 measurements taken about 1 mm. from the entrance of the optic nerve.







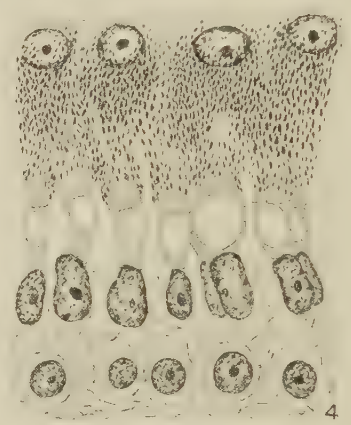


Fig. 1 A portion of the retina of Chrysemys picta. Animal kept in darkness for 24 hours.

Fig. 2 A portion of the retina of Chrysemys picta. Animal, after having been kept in darkness for 24 hours, placed in sunlight for 6 hours.

Fig. 3 A portion of the retina of Chrysemys. From an individual with cut optic nerve, kept in darkness for 24 hours, followed by a 7 hour exposure to sunlight.

Fig. 4 A portion of the retina of Chrysemys. From an individual with cut optic nerve and kept in darkness for 24 hours.

All of the figures were drawn with the aid of the camera lucida, and with Leitz oil immersion $\frac{1}{12}$ objective and ocular 4, giving an approximate magnification of 1340 diameters. The figures were then reduced $\frac{1}{3}$ for publication so that as they appear they have a magnification of about 890 diameters.

All of the drawings were made at about 1 mm. distance from the entrance of the optic nerve.

The comparisons of light and dark eyes of Sceloporus show that a pigment migration also takes place here. The average of measurements indicate that the extent of this migration is 3.1μ (table 2).

TABLE 2[1]

ANIMAL	DIST. FROM EXT. LIMIT. MEMB. TO CHOROIDAL EDGE OF PIGMENT EPITHELIUM	DIST. FROM EXT. LIM. MEMB. TO OUTER SEG. OF CONE	DIST. FROM EXT. LIM. MEMB. TO NEAREST PIGMENT NEEDLE
Sceloporus			
Dark..........	22.5μ	14.0μ	9.0μ
Light..........	19.1μ	12.5μ	5.9μ

[1] Based on 10 measurements as in table 1.

Not only does the pigment migrate but the cones also show a contraction in light. In table 1 the results of measurements are shown. In the first place in the dark eye the distance between the external limiting membrane and the choroidal edge of the pigment epithelium is 5μ more than in the light eye. But this does not represent the amount of contraction of the cones, for as will be seen (column 2) the average length of the cones in the dark eye is only 2.3μ longer than in the light eye. From this it is evident that the light not only causes a shortening of the cones but a flattening of the pigment epithelial cells of, on the average, 2.7μ.

This flattening of the epithelial cells in the tortoise retina is in line with the results of others. Angelucci ('84 and '94) pointed out that in the illuminated eye of the frog a shortening of the epithelial cells in the direction of the axis of the rods was to be observed. Chiarini ('04 and '06) also saw this change in the retinal epithelial cells of the representatives of the five classes of Vertebrates which he examined. And Pergens ('96) noted the same thing in Leuciscus.

In addition to this change in form of the epithelial cell there has also been observed a change in the position of the nuclei of these cells. Pergens ('96) found that after illumination the nuclei of the pigment cells of Leuciscus were further forward than in dark eyes. Angelucci ('94) also observed this change of position in the frog. Garten ('07 b) on the other hand, although he observed the differences in position of the nuclei of the epithelial cells of Abramis could find no constant differences due to the effect of light and darkness.

Concerning the third of the effects of light on the retina in which we are interested, namely changes in form, position and the ability of the ganglion cells and of the nuceli of the inner and outer granular layers to stain there has grown up a comparatively large literature. This has been excellently reviewed by Garten, who has in addition given a table in which the results of different investigators are shown.

We will first take up the effects of light on the nuclei of the cones. In Garten ('07 b, pp. 18–23) will be found a very good summary of what has been done. Czerny ('67), Gradenigo ('85), Angelucci ('94), and Chiarini ('04) in the frog; Pergens ('96) in Leuciscus rutilis; and Chiarini ('06) in the lizard found that light caused the cone nuclei to become longer and narrower. In addition, Birch-Hirschfeld (' 06) found that light caused a difference in the volume of the cone nuclei of the pigeon, in that they are smaller and narrower. Birch-Hirschfeld further found in the pigeon that light caused the cone nuclei to approach nearer to the external limiting membrane.

Light was found to decrease the power of the outer granules to stain by Pergens ('96, '97, and '99) in Leuciscus rutilis, Mann ('94) in the dog; Birch-Hirschfeld ('00) in the dog and cat, and ('06) in the pigeon (very slight), Sgrosso ('05) in the frog, and Garten ('07, p. 23) in Cercopithecus, Macacus rhesus, fishes (Abramis and Leuciscus), Salamandra, frog and the owl. Chiarini ('04 and '06), however, found that in Leuciscus any difference between the ability of the dark and the light eye to stain was very uncertain, and that in the frog, lizard, crow and dog there was absolutely no difference.

We may pass now in the same way to a brief review of the results that have been obtained concerning the effect of light on the form and stainability of the inner granules and of the ganglion cells. Mann ('95) found that in the dog, illumination of the eye for 12 hours caused a decrease in the stainability of the inner nuclear layer and a decrease in the Nissl substance of the protoplasm of the ganglion cells. Bach ('95) however, in the rabbit could find no differences in the stainability of the inner nuclear layer or of the ganglion cells in light and dark eyes. Birch-Hirschfeld ('00) however, did find differences between the light and dark eyes of rabbits and of dogs. The nuclei of the inner layer in the dark eye are rounder, in the light eye more oval. In the ganglion cells the Nissl bodies in the light eye have indistinct boundaries and with the protoplasmic background very diffuse. In the dark eye, on the other hand, the Nissl bodies possess sharp, distinct outlines. Chiarini ('04) found in Leuciscus, no decrease in chromatin in the inner nuclear layer after illumination, and in the ganglion cells hardly noticeable changes. Also later ('06) in the inner layer of reptiles, birds and mammals he found no differences between light and dark retinae. In Lacerta, however, he observed a slight decrease in the Nissl bodies of the ganglion cells, in Corvus a decided decrease, and in the dog again a slight chromatolysis.

Schüpbach ('05) found no differences, neither in the inner layer nor in the ganglion cells, between light and dark eyes of pigeons. But Birch-Hirschfeld ('06) was able to demonstrate clearly that the ganglion cells showed a distinct decrease in the number of the Nissl bodies and an indistinctness of their boundaries in the light eye. Carlson ('04) has shown the same for another bird—Phalacrocorax penecillatus, and finally Sgrosso ('05) found in the frog that the inner nuclear layer showed differences in stainability in the light and dark eye.

In order to make observations on these matters, particularly on the stainability of the nuclei, it was necessary to use some other methods of fixing, etc., than had been employed for the study of pigment migration, and of cone contraction. For this purpose the following method was finally decided upon, and gave excellent results. Fixation in warm concentrated sublimate for 5 hours. Removal of sublimate with iodine in 70 per cent alcohol, further dehydration, and infiltration by the chloroform paraffin method. Sections were cut 8μ thick, stained in eosin and toluidin blue, rapidly dehydrated, cleared in xylol and mounted in damar. In order to insure the same amount of fixation, staining, decolorizing, etc., light and dark eyes were fixed in the same vessel, care being taken that they were distinguishable. Further, after sectioning, alternate rows of dark and light eyes (two of each) were placed on the same slide so that there could be no doubt of their similarity of treatment.

In the first place, the cone nuclei of both the tortoise and of the lizard are, for the most part, lengthened and narrowed by illumination. This change is, however, not very great and there is room for doubt owing to the great variability in the shape of these nuclei both in the light and in the dark eye. As far as their ability to stain is concerned the nuclei of the outer granular layer in the dark eye seem to be slightly more deeply stained than those of the light eye, but as far as the nuclei of the inner granular layer is concerned, no differences can be found. Neither could any changes in form of the inner nuclei be noticed after long illumination nor could any changes in the form and volume of the ganglion cells be observed. These latter cells, however, do show marked and clear differences between the light and dark eyes (figs. 8 and 9). Figure 8 represents three ganglion cells from a dark eye. By comparing it with figure 9 which is from a light eye, the differences can be observed. Not only is the amount of chromatin reduced but the Nissl substance has decreased in amount. Under the microscope these differences can be seen very distinctly, and it is possible to pick out the light and dark eyes by the comparative amount of chromatin and of Nissl substance which they contain.

These results show that light causes a migration of pigment and a contraction of the cones, as slight as these may be, in the retina of the tortoises and lizard. Moreover, that the cone nuclei of the tortoises are probably narrowed and lengthened and that furthermore a diminution in the amount of chromatin and Nissl substance in the ganglion cells is brought about, so that they stain less darkly and more diffusely than in dark eyes. It was considered sufficiently interesting to attempt to find out whether some or all of these changes could be brought about in eyes which had either been enucleated or had had the optic nerve cut.

Angelucci ('78, p. 367) observed that when the optic nerve of a frog is cut the physiological changes of the pigment took place, thirty days after the operation, as in normal eyes. Hamburger ('88), Arcoleo ('90) and Fick ('01, p. 4) also found that when the optic nerve is cut the pigment changes induced by light and darkness took place as in the normal eye. In addition Engelmann ('85, p. 505) observed that when the brain of a frog is destroyed that the effect of light on the migration of pigment is still present. Movements of the cones have also been observed in eyes, the optic nerves of which have been cut, or which have been removed from the body. Hamburger ('89) found that, when he cut the optic nerve of a frog or removed the eye, contraction of the cones will take place when the eye is illuminated. And Dittler ('07) found in the isolated frog retina placed in salt solution that upon illumination the contraction of the cones takes place.

Experiments on the enucleated bulbus of the tortoise were without results. The same is true of retinae which were isolated in the manner described by Dittler for the frog. The experiments on eyes with the optic nerve cut, however, did yield rather interesting results. All the experiments were carried out on Chrysemys picta. The method of cutting the optic nerve is briefly as follows: Under deep ether anaesthesia a small wedge of bone was removed from the left side of the mouth beneath the eye. This was done by means of a long, very narrow saw. The small amount of bleeding consequent to the removal of the bone being stopped, the muscles were pulled to one side and cut, until the optic nerve could be seen. This was then cut by means of a fine pair of scissors. The total loss of blood was small and the animals quickly recovered. Out of eleven individuals operated upon only the first three died. The others after a few hours were active and seemed quite as fit for experimentation as normal animals. One effect of cutting the optic nerve, which was not always observed, was a slight enlargement of the pupil. Fick ('91, p. 3) states that after cutting the optic nerve of the frog the pupil is temporarily somewhat narrowed.

The tortoises were allowed sufficient time to recover from the effects of the operation before they were placed in light or darkness. Two operated animals were always used together for experiments and as controls two normal animals. All were placed in darkness for 24 hours, at the end of which time 1 operated and 1 normal individual were selected and placed in sunlight for 7 hours after which the eyes of all were removed and fixed as above described.

Upon examination and comparison of sections, it was seen that the pigment of the operated light eye had migrated forward and indeed further than it had in the normal eye of the same animal and in the eyes of the controls (figs. 2 and 3, and tables 1 and 3). Moreover that the cones had contracted about as much as in the normal eyes (see tables 1 and 3).

TABLE 3[1]

ANIMAL	DIST. FROM EXT. LIMIT. MEMB. TO CHOROIDAL EDGE OF PIGMENT EPITHELIUM	DIST. FROM EXT. LIM. MEMB. TO OUTER SEG. OF CONE	DIST. FROM EXT. LIM. MEMB. TO NEAREST PIGMENT NEEDLE
Chrysemys			
Operated dark	29.6μ	24.8μ	4.7μ
Operated light	23.5μ	22.3μ	2.7μ

[1] Based on 10 measurements as in tables 1 and 2.

Now when the dark eyes of the operated and the control animals are compared, it is found that the pigment of the operated eyes is further forward than in the control eye of the same animal and in the eyes of the control animals. In fact the position of the pigment in the operated dark eye is about as far forward as it is in the normal light eyes (figs. 2 and 4, and tables 1 and 3). These results also show that the cones contract in light when the optic nerve is cut (table 3). Moreover, by comparing tables 1 and 3, it will be seen that the cones are longer in the operated dark eye than in the normal dark eye. These results would seem to be in line with those of Herzog ('05) who found that destroying the central nervous system of the frog brought about in the dark eye examined immediately or 24 hours later, a maximal forward migration (light position) of the pigment and a maximal stretching (dark position) of the cones. It would seem as if a tonus, not only of the cones, but of the pigment epithelial cells, was removed or destroyed and that they both relaxed. Dittler ('07) and Garten ('07) were not able to corroborate Herzog's results. Garten, on the other hand, found that when the optic nerve of a frog is cut a marked stretching of the cones may take place, but does not mention any effect on the pigment, while Dittler (p. 306) says that destruction of the central nervous system, cutting of the optic nerve and enucleation of the bulbus has not the slightest effect upon the length of the cones.

Since the migration of the pigment and the contraction of the cones is relatively so small in amount, it was considered interesting to find out whether a greater amount of migration and of contraction could not be induced by means of other stimuli. The most natural stimulus to try was an electric current and therefore this was done, both induced and constant currents being used.

The experiments and results of stimulating the retina with an induced current will first be considered. The eyes, both dark and light, were removed as quickly as possible and placed in a watch glass containing a little 0.7 per cent salt solution. In one series of experiments both electrodes were placed on the optic nerve, in another, one was placed on the nerve and the other on the cornea. Stimuli of moderate strength were used and for a period of from 10 to 15 minutes. Upon examination of sections of such eyes it is seen that the pigment undergoes migration, but also a pronounced bunching and massing up in various places. The cones are also affected by the induced current being broader and shorter than normally the myoid being considerably contracted (figs. 6 and 10 and table 4). All of the cones did not show this effect, some of them appearing like normal dark cones and similar in measurements.

Engelmann ('85, p. 508) found that stimulation of the intact or enucleated dark eye of the frog with an induced current of moderate strength, produced the light condition of both cones and pigment.

When a constant current is passed through the tortoise eye much more definite and pronounced effects are obtained than with an induced current. The eyes, after being removed, were placed in a long trough containing tap-water to which a little salt solution had been added. The current (15–20 M. A.) was then passed from one end to the other and for from 15 to 30 minutes. Both light and dark eyes were again used and the current in some was passed from the posterior to the anterior of the eye (centrifugal), and in others, in the opposie direction (centripetal).

The examination of sections of eyes treated in this way show that in all cases a migration of the pigment takes place (fig. 5). This represents a typical case and in table 4 are given the average of a series of measurements.

TABLE 4[1]

ANIMAL	DIST. FROM EXT. LIMIT. MEMB. TO CHOROIDAL EDGE OF PIGMENT EPITHELIUM	DIST. FROM EXT. LIM. MEMB. TO OUTER SEG. OF CONE	DIST. FROM EXT. LIM. MEMB. TO NEAREST PIGMENT NEEDLE
Chelopus insculptus			
Induced current eye.....	30 μ	19.4μ	5.0μ
Constant current eye.....	33.7μ	25 0μ	3.4μ

[1] Based on 10 measurements.

A current which has been passed through the eye for 15 minutes is as effective as one which was allowed to pass for 30 minutes and presumably weaker currents of shorter duration would give the same results. In a few cases the retinae through which the currents had been passed for thirty minutes showed a partial breaking and tearing apart, particularly of the inner and outer nuclear layers.

The effects of a constant current on the cones is also very striking. Again the cones are much broader than normally, but instead of being contracted as after stimulation with the induced current they are much elongated (fig. 11, and table 4). The cone nuclei are not usually affected in any constant way and are either round or oval as is ordinarily the case. In a few cases however they seem to be broken down and drawn out and up into the myoid (fig. 5).

It has been known for some time that the melanophores of the skin in many animals are caused to contract when stimulated with an induced current and to expand when stimulated with a constant current (Laurens '15, p. 609). It would seem that the same is true of the pigment cells of the retinal epithelium.

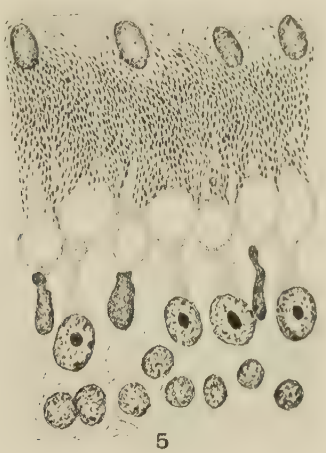


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Fig. 5 A portion of the retina of Chelopus insculptus showing the effects of passing a constant current of 18 M. A. for a period of 15 minutes through the eye. (Current passing from the cornea towards the optic nerve).

In all four cases in which the current was passed from the cornea to the optic nerve the pigment cell nuclei were changed in shape and position (fig. 5). Instead of being roundly oval with the long axis parallel to the choroidal limit of the cell, they are narrowed and lengthened and with the long axis at right angles to the usual position. This condition of affairs was never found when the current was passed in the opposite direction and would seem to indicate a polar effect of the current.

DISCUSSION AND CONCLUSIONS

In the retina of the tortoises (Chelopus insculptus, Chelopus guttatus and Chrysemys picta) and of the lizard (Sceloporus undulatus) pigment migration and cone contraction undoubtedly take place, however slight they may be as compared with the conditions observed in other groups of animals. Garten ('07 a and '07 b) has advanced a theory to explain the migration of the pigment and the contraction of the cones. His theory necessitates the assumption that there is no migration of pigment in the eyes of animals possessing a purely rod retina (skates and sharks) or a purely cone one (reptiles in general). Garten's theory in brief is as follows: one must assume that, were it not for the optical isolation of the visual elements by the pigment which migrates forward in the eyes of most of the lower vertebrates, a great deal of light would be scattered in all directions, on account of the large ellipsoids (fishes) and the strongly refractive oil-drops (many Amphibians, Reptiles and Birds) which would stimulate the neighboring rods and cones. In the narrow slender rods, however, total internal reflection prevents this dispersion of light, which is borne out by the fact that in pure rod retinae the pigment is entirely lacking from the pigment epithelium. On the other hand in a pure cone retina with strongly refractive oil drops, the pigment in both light and dark eyes must be forward in position (that is, covering the outer segments and the oil drops). By means of the stretching of the cones in darkness or in faint light, which in many of the lower animals is accompanied by a contraction of the inner segments of the rods, there is presented for faint light stimulation, a cone-free purely rod layer, which does not need the pigment, which therefore moves backward thus enabling oblique light to enter the rods. On the other hand the contraction of the cones produced by bright light, which is accompanied by stretching of the rods, similarly leads to a forward migration of the pigment which surrounds the cone outer segments.

This is the part of Garten's theory which is of interest to us. It cannot be denied that the tortoise and lizard retina contains only cones and no rods. This has been shown, not only morphologically but physiologically as well, in that it is impossible to demonstrate the presence of visual purple (see Garten '07 b, p. 148 and Hess '10, p. 281). Nevertheless, pigment migration and cone contraction do take place, however slight in amount. It is perfectly true that the outer segments and the oil drops are always covered with pigment, but when bright light (sun-light) is thrown on the retina the cones contract and the pigment migrates forward.

It cannot be argued, if we accept Garten's theory, that there is any physiological need for a moving backward of the pigment and a stretching of the cones in faint light since there is only one kind of visual cell. But, on the other hand, it must be assumed that the pigment and the cones are sensitive to light and respond to its stimulus, the one moving forward, the other contracting. Dittler ('07 a and '07 b) thinks that the cone myoid of the frog is not itself sensitive to light but that its contraction is brought about by a metabolic product which is set free by the activity of the retina under the influence of light. Garten ('07, p. 96) supports this theory and thinks that the migration of pigment may also be bound up in some way with this chemical stimulus effect. Now, if this be true for the retina of the frog, there is no reason, simply because the tortoise and lizard retina have only cones, to assume that it is not true for the retina of these animals.

Gaupp ('04, p. 815) is of the opinion that in the migration of the pigment in the protoplasmic processes between the visual elements we have a process very similar to that seen in the melanophores of the skin, where the pigment streams back and forth in the processes of the cells. We have seen in the tortoise eye where the optic nerve has been cut that migration of the pigment, as well as contraction of the cones, still takes place, which is evidence that both the pigment cell and the cone are also directly sensitive to light. Garten's objection that there has been no proof that pigment migration in the retina may be brought about by the direct effect of light because chemical stimuli could possibly play a part has, it seems to me, no weight, for the simple reason that that is probably just what does take place, not only in the retinal pigment epithelium but in the skin melanophores as well, when stimulated by light. Light causes chemical changes which influence the pigment cells and causes the protoplasm, in which the pigment is carried, to stream.

SUMMARY

1. The retinae of the tortoises, Chelopus insculptus, Chelopus guttatus and Chrysemys picta and of the lizard, Sceloporus undulatus contain no rods.

2. In both the tortoise and lizard double, as well as single, cones occur (figs. 6 and 7).

3. Light causes a migration of the pigment and a contraction of the cones in both the tortoise and the lizard retina, the extent of migration in the tortoise averaging 3.6μ and in the lizard 3.1μ. The extent of the contraction of the cones in the tortoise averages 2.3μ. The pigment epithelial cells also flatten in light to the extent of about 2.7μ.

4. Light probably causes the cone nuclei to lengthen and become narrower. Illumination decreases slightly the ability of the outer nuclei to take on stain, but has no effect on the form nor on the ability of the nuclei of the inner granular layer to stain.

5. Illumination has no effect on the form and volume of the ganglion cells, but it reduces the amount of chromatin and Nissl substance so that the cells stain less darkly and more diffusely (figs. 8 and 9).

6. Light causes the pigment to migrate and the cones to contract in the tortoise retina after the optic nerve is cut (fig. 3).

7. Stimulation of the enucleated bulbus with an induced current of moderate strength causes a forward migration as well as a bunching and massing of the pigment. It also causes a slight broadening and contraction of the cones (fig. 10).

8. Passing a constant current of 15 to 20 M. A. through the eye either centrifugally or centripetally for a period of 15 minutes brings about a marked migration of the pigment and in addition a broadening and stretching of the cones. When the

current is passed from the cornea towards the optic nerve (centripetally), a polar effect on the epithelial cell nuclei is observed in that the nuclei are rotated so that the long axis of the nuclei are at right angles to their usual position (fig. 5).

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PLATE 1

EXPLANATION OF FIGURES

All of the figures were drawn with the aid of the camera lucida, and with Leitz oil immersion $\frac{1}{12}$ objective and ocular 4, giving an approximate magnification of 1340 diameters. The figures were then reduced $\frac{1}{3}$ for publication so that as they appear they have a magnification of about 890 diameters.

All of the drawings were made at about 1 mm. distance from the entrance of the optic nerve.

6 Cones of Chrysemys. Animal kept in darkness for 24 hours.

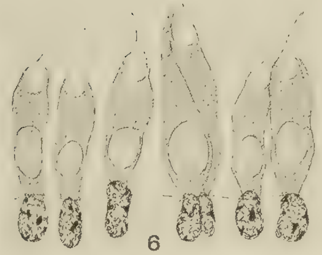
7 A portion of the visual layer of an eye of Sceloporus undulatus showing double cones and the two types of single cones.

8 Three ganglion cells from the retina of Chrysemys picta. Animal kept in darkness for 24 hours.

9 Three ganglion cells from the retina of Chrysemys picta. Individual held in darkness for 24 hours and subsequent exposure to sunlight for 6 hours.

10 Cones from the retina of Chrysemys picta showing the effects of stimulation with an induced current for 10 minutes.

11 Two cones from the retina of Chelopus insculptus showing the effects of stimulating with a constant current of 18 M. A. for 30 minutes.



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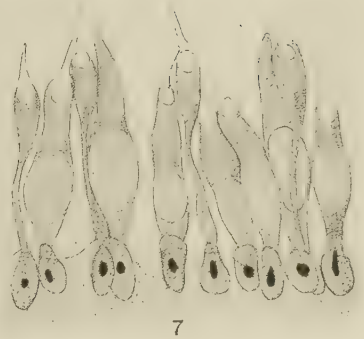

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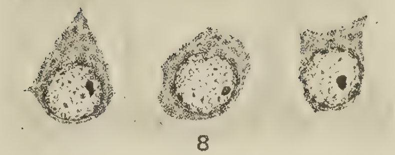

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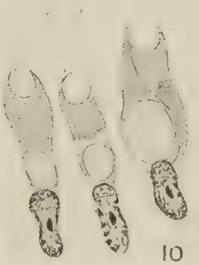

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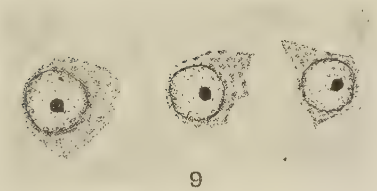

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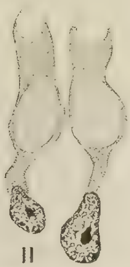

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THE PERIODIC REORGANIZATION PROCESS IN PARAMAECIUM CAUDATUM

RHODA ERDMANN AND LORANDE LOSS WOODRUFF
Osborn Zoölogical Laboratory, Yale University

THIRTY-FIVE FIGURES (SEVEN PLATES)

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I. INTRODUCTION

In previous papers[1] there have been presented the morphological and physiological details of a new reorganization process in the life of Paramaecium aurelia, and the statement that the phenomenon occurs also in Paramaecium caudatum. The present paper is a study of this reorganization process, which we term endomixis, in the latter species of Paramaecium. We shall in this paper confine ourselves chiefly to a description of the facts observed and to a very brief discussion of the theoretical bearings, leaving for consideration at another time, when the details of this reorganization process in other species of Protozoa have been discovered, the more or less academic discussion as to the exact classification of this process among phenomena of Entwicklungserregung. However, we may state at once, in view of the somewhat premature criticism by R. Hertwig of our work as presented by us in an avowedly preliminary paper ('14, II), that we are convinced, after such a careful consideration as any paper by Hertwig demands, that not a single objection which he raises is well founded. We are also convinced that the theoretical interpretations which Calkins ('15) advances in regard to our results are not warranted by the facts so far at hand. We take, then, in their entirety the facts and discussion as presented in our complete paper ('14, III) as the starting point for our present communication.

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[1] Woodruff and Erdmann ('14, I): Complete periodic nuclear reorganization without cell fusion in a pedigreed race of Paramaecium. Proc. Society for Experimental Biology and Medicine, vol ii, Feb. 18. (Preliminary paper.)

Erdmann and Woodruff ('14, II): Vollständige periodische Erneuerung des Kernapparates ohne Zellverschmelzung bei reinlinigen Paramaecien. Biol. Centr. Bd. 34. (Preliminary paper).

Woodruff and Erdmann ('14, III): A normal periodic reorganization process without cell fusion in Paramaecium. Journal of Exper. Zoölogy, vol. 17, no. 4, Nov. (Complete paper.)

II. MATERIAL AND METHODS

The material used in the present study has been derived from pedigreed races of Paramaecium caudatum which have been bred in exactly the same way as described in our work on Paramaecium aurelia, and the reader is again referred to that paper for details ('14, III, p. 432).

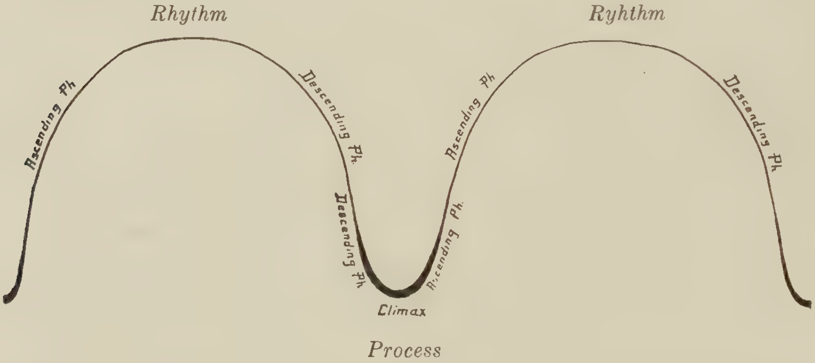
From our study of several races of Paramaecium caudatum it seems clear that this species is less well adapted than Paramaecium aurelia to withstand the conditions necessary for pedigreed culture work. Whereas all the races of Paramaecium aurelia which we have studied have survived indefinitely under the daily isolation slide method of pedigreed culture manipulation, most of the Paramaecium caudatum races have sooner or later refused to divide under these conditions. As will be discussed in detail later, the races on the slides can undergo the reorganization, perhaps two or three times and then at the next onset of the phenomenon, about 90 generations later, are unable to carry it to proper completion and die. If, however, the animals are placed in tiny tubes of culture medium, instead of on slides, so that the volume of culture medium is somewhat greater, but not large enough to render it impossible to control them daily and so detect conjugation if it occurs, then the animals apparently can live indefinitely. This result is, in general, in accord with the earlier observations of Woodruff ('11c, p. 64) though at that time he used somewhat larger volumes of medium to save the Paramaecium caudatum and so was not able to positively prove that conjugation did not occur.

It has seemed best to consider this point in justification of our resorting to material from tiny tube cultures for certain stages of the reorganization process. But we would emphasize that many of the stages of this process in Paramaecium caudatum, as all of the stages of the process in Paramaecium aurelia, have been secured from daily isolated pedigreed animals so that it is absolutely positive that the phenomenon under discussion occurs in caudatum as in aurelia at clearly defined periods. To repeat—we have shown, by the isolation slide method, the physiological characteristics of the reorganization process in the recurrent rhythms of the division rate, and the accompanying cytological changes of this process to the climax at the low points of the rhythms. The completion of the process in isolated slide animals is evident from the completion of the rhythms in many cases. But owing to the longer rhythmic periods in Paramaecium caudatum as compared with Paramaecium aurelia, and owing to the lesser viability of the former species and the smaller number of generations over which its reorganization process extends, we did not secure all of the later cytological changes from isolated slide animals but resorted to carefully controlled 'tube' animals as already described. We will grant that the original discovery of the reorganization process in Paramaecium caudatum would have been more difficult than it was in Paramaecium aurelia because of the longer rhythmic period in the former species and its less marked viability under the artificial slide pedigreed method during the crucial stages of the phenomenon.

III. THE CYTOLOGICAL PHENOMENA OF THE REORGANIZATION PROCESS IN PARAMAECIUM CAUDATUM

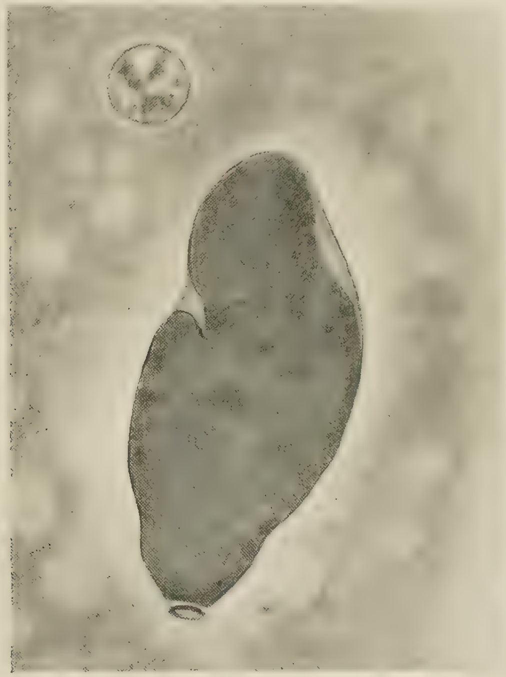
The complete reorganization process without cell fusion which occurs in Paramaecium caudatum, as in Paramaecium aurelia, synchronously with the rhythms, presents essentially the same fundamental cytological features as those we have described in the latter species ('14, III, pp. 436–473).

We shall, therefore, present merely a general summary of the phenomenon in Paramaecium caudatum and point out the differences which could be traced in the morphological features. In accordance with our previous investigation we term the three phases into which the reorganization process (endomixis) naturally resolves itself as descending phase, climax and ascending phase (text figure 1).



Text fig. 1 Diagram illustrating the relation of the reorganization process to rhythms. (Woodruff and Erdmann, '14, III.)

A. The descending phase. The micronucleus of a typical Paramaecium caudatum which is not undergoing the reorganization process or normal vegetative division is situated in a slight depression of the macronucleus. Text-figure 2 (p. 63) shows an animal shortly after a typical vegetative *division* in which the micronucleus has not yet reached its accustomed position. The structure of the resting micronucleus between two vegetative cell divisions is shown in figure 1, p. 85. The position of the micronucleus in figure 2 has not changed but its morphological structure is characteristic of beginning reorganization. As this phenomenon approaches the micronucleus fails to take up its accustomed place, and remains free in the cytoplasm, while the chromatin of the micronucleus seems to assume the form of short bands. Figures 3 and 4 show two animals in the 203d generation from different sub-lines of Culture Y just when the process of reorganization begins—the formation of bands of chromatin, and the wandering of the micronucleus in the cytoplasm, indicating the definitive onset of the process. This wandering of the micronucleus has been described by us in the reorganization of Paramaecium aurelia ('14, III, figs. 3, 4, 5).



Text fig. 2 Culture Z, Line I, 38th generation, October 12, 1914

Projections of the macronucleus which extrude at a very early stage of the process of reorganization in Paramaecium aurelia have not been seen in our races of Paramaecium caudatum.

Here the macronuclear surface loses its smooth appearance while clefts and wrinkles appear in the stained preparations thus indicating that in the living cell the distribution of the chromatin is not homogeneous in the macronucleus (figs. 4 and 7).

The micronucleus begins to swell shortly after emergence from its depression in the macronucleus. Figure 5, shows the chromatin arranged at the equator of the micronucleus in a band of granules while one pole is nearly devoid of chromatin. (Compare Calkins and Cull '07, fig. 1). The swelling continues until the so-called sickle stage appears. Figure 8 shows a more elongated micronucleus than the one shown in figure 5. The formation of the 'sickle' is completed in an animal from the 202d generation, Culture Y, Line III g (fig. 7). The paucity of the chromatin in the portion of the micronucleus which protrudes from under the macronucleus is remarkable; no distinct granules or threads being recognizable as the chromatin is apparently homogeneously distributed throughout the dividing micronucleus at this stage. The formation of chromatin bodies has begun in the macronucleus. Maupas, Hertwig and Hamburger described, in the different species of Paramaecium which they investigated, the occurrence of the 'sickle' stage before the onset of the reduction divisions preceding *conjugation*. The 'sickle' stage changes in conjugation into the first reduction spindle as described in detail by Calkins and Cull, '07, p. 383, in Paramaecium caudatum. In the reorganization process of Paramaecium caudatum we secured an animal from the 184th generation with the very characteristic dumb-bell form of the micronuclear division spindle (fig. 6). The 'dumb-bell' micronuclear division stage in the reorganization process resembles closely the stage which Calkins and Cull ('07, fig. 15) interpret as the telophase of the second maturation division in conjugation. In the reorganization process of Paramaecium caudatum there is a slight difference in size of the arising micronuclei as is indicated in the cell shown in figure 6. The amount of chromatin at the two ends is apparently different. It is possible that the arising micronucleus which is nearly devoid of chromatin is the one whose products after the next division are doomed to degeneration.

Calkins and Cull do not describe a 'dumb-bell' formation in the first reduction division in conjugation, but we must interpret our figure as the first 'reduction' division of the reorganization process because no trace of other micronuclei are in the cell. An animal in the second 'reduction' division is shown in figure 10. Two micronuclei are dividing. In one the equatorial band of chromatin is intact while in the other it is divided. The macronucleus, fragmentated into two parts, is rapidly losing its chromatin.

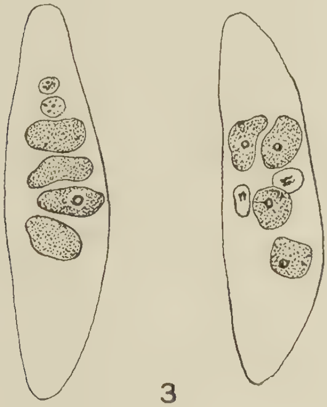
The breaking up of the macronucleus begins relatively late in Paramaecium caudatum and generally does not extend through several generations as we found to occur in the descending phase of the process in Paramaecium aurelia (Compare '14, III, pl. 1). The expulsion of chromatin bodies (figs. 14, 15 and 16) or the breaking of the macronucleus into two or more pieces (figs. 9, 10 and 11) seem to be a rapid process in the life of the caudatum cell. Culture Z, 91st generation (fig. 13) does not show the slightest trace of chromatin bodies, but in the 93d generation (fig. 14) there are many of them in the cytoplasm. These chromatin bodies, however, do not have the definite condensed appearance so characteristic of those in Paramaecium aurelia, but are relatively pale and indistinct and seem to suddenly appear in nearly maximum numbers. This possibly may be accounted for by the fact already mentioned that in Paramaecium caudatum finely divided chromatin material, in certain cases at least, streams from the macronucleus into the cytoplasm. This leaves a paucity of chromatin for the chromatin bodies and may change the tension within the macronuclear membrane so that the chromatin bodies are formed rapidly and thus appear suddenly. Figure 13 gives a good idea of this phenomenon, as does also figure 6 in which the macronucleus is partly devoid of chromatin while no chromatin bodies are visible.

This breaking up of the macronucleus into large pieces was observed in animals from small tube cultures (fig. 10) as well as in isolated animals (figs. 9 and 11) as already described. These animals cannot be dividing specimens because in normal vegetative divisions the macronucleus is greatly elongated and breaks only at one point when the cytoplasm itself has nearly completed division ('14, III, fig. 7).

To summarize: The loss of chromatin occurs either by the extrusion of chromatin bodies (Paramaecium caudatum and Paramaecium aurelia); by extrusion of small granules from the macronucleus (Paramaecium caudatum); by breaking up of the old macronucleus into two or more large pieces (Paramaecium caudatum; Paramaecium aurelia, Hertwig '89, p. 74). The result—the total destruction of the individuality of the macronucleus—is the same in each case.

Extruded chromatin bodies were figured by Calkins '04 (fig. 16) in Paramaecium caudatum and these we have compared with certain stages of Paramaecium aurelia at the beginning of the ascending phase of the process of reorganization ('14, III, p. 484, text figs. 20 from our own preparations, and 19, copied from Calkins). Calkins does not mention how the nuclear fragmentation has been effected but this probably has occurred in the same way as we have described in our cultures of Paramaecium aurelia (Woodruff's main culture I, and Erdmann's culture B) and in some animals from our culture Y of Paramaecium caudatum ('14, III, fig. 34), because the morphological features are identical. Those cultures of Paramaecium caudatum, in which the macronucleus breaks into large pieces, present in the further stages of macronuclear degeneration a different cytological appearance as is shown in an animal from about the 82d generation, culture Y (fig. 8). Here the chromatin is distributed in the cell without first being condensed into spherical chromatin bodies. The macronuclear membrane is torn and its contents are intermingling with the cytoplasm. This general type of fragmentation of the macronucleus has been described with different interpretations by several authors, for example, Kasanzeff '01, working under R. Hertwig; Popoff '07, figure 18, plate 4; Popoff '09, figure 26, plate 2; Calkins '04, figure 8, plate 1 and figure 11, right animal, plate 2; and Hertwig '14, p. 568. All these authors considered these changes as depression phenomena which, according to Popoff, have close resemblance to phenomena characteristic of the onset of conjugation.

Now Hertwig '14, reviewing and commenting on our preliminary paper ('14, II) on the reorganization process of Paramaecium aurelia, in the light of his theoretical beliefs in regard to depression in Protozoa, shows in his text figure 3, animals 4 and 5 (copied as text fig. 3 in this paper) the breaking up of the macronucleus together with the formation of two micronuclei. These stages correspond somewhat to our stages (figs. 8, 9, 10 and 11) at the end of the descending phase of the reorganization process when the first or second 'reduction' division has occurred. But it is clear that Hertwig has confused two different series of phenomena: animals with hypertrophied macronucleus and not clearly discernible changes in the micronucleus, with those of the reorganization process which we have described as endomixis (cf. text fig. 3, copied from Hertwig). We have never found in the thousands of isolated animals studied that the macronucleus was enlarged by any means to such a degree as Hertwig figures in his text figure 3, animals 1, 2, 3, from which we have copied the second animal as our text figure 4.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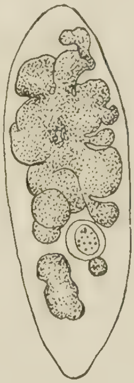
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Text figs. 3 and 4 Paramaecium caudatum. From Hertwig, '14, after Kasanzeff.

The crucial stage, i.e., the total reorganization of the nuclear apparatus with the formation of the macronuclear anlagen, could not have been determined as a phase of the reorganization process either by Hertwig, Kasanzeff or Popoff in their *mass* cultures. However, Hertwig's suggestion that "Woodruff und Erdmann könnten somit die Stadien als Beweise für eine vorausgegangene Parthenogenese in Anspruch nehmen" is a partially right prediction, provided one substitutes for 'parthenogenesis,' reorganization process -endomixis! Since Kasanzeff did not find stages of the ascending phase, that is the formation of the macronuclear anlagen, in Paramaecium caudatum mass cultures, Hertwig still believes that the described phenomena are depression conditions and not stages of the reorganization process, though he himself described in Paramaecium aurelia isolated stages of a process which he called 'parthenogenesis.'[2]

We wish to emphasize that in a mass culture it is impossible to know the ancestry of the individual cell. It is clear that from a mass culture so-called depression stages may be either animals which are about to begin the reorganization process, or animals which have just undergone conjugation, or abnormal animals due to some exigencies of the environment. From material of this sort, in view of the fact that the reorganization was not known previous to our work, there has been drawn into the literature on infusorian life histories the long series of atypical animals as evidence of depression. However, by pedigreed culture methods we have resolved this heterogeneous material into its component parts and have shown for both Paramaecium aurelia and Paramaecium caudatum that *many* of the so-called depression phases are normal stages in a complex reorganization phenomenon.

B. Climax. Culture Y of Paramaecium caudatum we had under observation from February 18, 1914, to June 9, 1914, and during this time the culture attained the 207th generation. The process of reorganization occurred at about the 18th, 136th and 203d generation (table 1). Line III which had successfully undergone the process at the 136th generation lived to the 192d generation, just at the onset of the next reorganization period. That the phenomenon was imminent is evident also from Subline III fa, in which it appeared at the 202d generation. From a close analysis of the division rate, and of the longevity of the component lines of Culture Y (table 2) it is apparent that of 4 lines and 13 sublines, 13 did not undergo a division for at least twenty-four hours, and in some cases for forty-eight hours, before their death. Death occurred in these lines either 'naturally' or as a result of the animals being killed for study since they were dividing slowly. Two lines or sublines died naturally without a lowering of the division rate, ten were purposely killed and four died after a lowering of the division rate. Sub-line III a,

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[2] We have not called the reorganization process 'parthenogenesis' and have not introduced even our new term 'endomixis' in our preliminary papers ('14, I and '14, II) because we wished to wait until our complete data was presented ('14, III) so that they could be discussed in their entirety. However, in our brief paper ('14, II) an error is present on page 495, owing to the fact that the printer misunderstood our directions and that we were unable to revise the proof on account of the war. The sentence "Diese Parthenogenese hier ist ein Sexualakt." is a remnant of a paragraph which appeared in the manuscript but was entirely deleted in the proof, but the printer in some way left this one isolated sentence for the complete paragraph!

TABLE 1

Showing the occurrence of the reorganization process in Culture Y

PERIODS =	1—90 GEN.	90—180 GEN.	180—270 GEN.	REMARKS
I. 1-59				Died
II. 1-107	18			Died
III. 1-192		136	191	Died
III a.* 120-130		139		Killed in descending phase
III b. 143-163				Died
III c. 161-163				Killed
III d. 161-165				Killed
III e. 165-169				Died
III f. 167-184		137		Killed
III fa. 169-203			202	Killed in descending phase
III fb. 169-206			203	Died
III g. 192-207			204	Died
IV. 1-89	26			Killed
IV a. 85-115				Killed
IV aa. 93-143		139		Killed in ascending phase
IV ab. 108-114				Died
IV ac. 120-137		137		Killed in descending phase

*Designated Line 2 in our 1914 (III) paper.

TABLE 2

Culture Y

LINE	GENERATIONS	DIVISIONS	FATE
I	59	None in 48 hours	Died
II	107	Two in 24 hours	Died
III	192	None in 24 hours	Died
III a	139	None in 24 hours	Killed
III b	161	One in 24 hours	Died
III c	163	None in 24 hours	Killed
III d	165	None in 24 hours	Killed
III e	169	Two in 24 hours	Died
III f	184	None in 24 hours	Killed
III fa	202	None in 24 hours	Killed
III fb	206	None in 24 hours	Died
III g	207	None in 24 hours	Died
IV	89	None in 24 hours	Killed
IV a	115	None in 24 hours	Killed
IV aa	143	One in 24 hours	Killed
IV ab	114	None in 24 hours	Killed
IV ac	137	None in 24 hours	Killed

III fa and IV ac, were killed in the descending phase of the process. Sub-line IV aa was killed after the climax.

Thus although cytological indications of the reorganization process were not observed in certain of the lines and sublines of Culture Y at the time of extinction, nevertheless this extinction was synchronous with the process as actually observed in other lines and sublines from the 136th to 143d generations, 184th to 191st and 202d to 209th generations. These periods clearly are critical ones in the life of this culture (table 2).

However our culture of Paramaecium aurelia was able to undergo the reorganization process frequently and with apparent facility under daily isolation methods, and therefore the number of lines which were eliminated by death during the phenomenon was relatively small (cf. '14, III, table 1, p. 462).

From all our work the conclusion evidently follows that before cytological signs of reorganization are discernible in the cell the physiological conditions for its onset are in evidence.

Culture Z was bred from September 20, 1914, and Culture M from January 7, 1915, to the end of our study. Culture Z underwent reorganization successfully in the 11th, 91st, 184th, 282d and 398th generations, and Culture M at the 89th generation, that is at intervals of about 90 generations, thus confirming our observation on Culture Y that the reorganization process occurs in Paramaecium caudatum at intervals of from 80 to 100 generations.

The difficulty of securing stages of the climax and of the ascending phase compelled us, as previously stated, to amplify our material of isolated pedigreed animals with small tube cultures seeded from our pedigreed cultures, just before reorganization was due to take place on the basis of our computation of the number of generations since its last occurrence and by the division rate. The most critical stage for the vitality of the race is when the individuality of the macronucleus is lost and the chromatin bodies are undergoing degeneration. The results of Calkins' extended experience with Paramaecium caudatum all indicate that the culture of Paramaecium caudatum in isolated lines on depression slides is sooner or later fatal. Woodruff's comparison, ('11 c, p. 60,) of pedigreed lines of Paramaecium caudatum with those of his main culture of Paramaecium aurelia showed clearly that it was impossible to breed indefinitely Paramaecium caudatum by the daily isolation method which was so highly favorable for Paramaecium aurelia.

TABLE 3

Showing the occurrence of the reorganization process in Culture Z

PERIODS=		1—90 GEN.	99—180 GEN.	180—270	270—360	360—450	REMARKS
I.	1-378	11	90	188	282		Killed
I a.	93-165						Killed
I b.	144-187			184			Killed
I c.	142-201			187			Killed
I ca.	171-260						Died
I d.	192-201						Killed
I e.	275-454						Discon'd
I f.	394-455					398	Discon'd
II.	1-92	91					Killed

The following tube cultures therefore were started from the respective isolated slide cultures as indicated above:

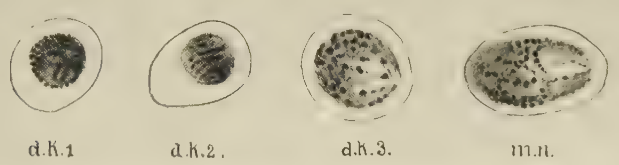
Culture Y	Tube culture a	March 26, 1914
	Tube culture b	April 1, 1914
	Tube culture c	April 3, 1914
	Tube culture d	April 20, 1914
	Tube culture e	April 22, 1914
Culture Z	Tube culture a	March 12, 1915
	Tube culture b	March 13, 1915

Continuing the discussion of the cytological changes of the reorganization process, fig. 18 shows an animal in the climax, from Culture Y about the 137th generation, without a complete macronucleus and with degenerating chromatin bodies as is indicated by their elongated form. This shape of the degenerating bodies we have frequently mentioned in our description of the climax and ascending phase of Paramaecium aurelia ('14, III, pl. 2). The definitive micronucleus (fig. 18), which by three subsequent divisions will form eight micronuclei from which the new nuclear apparatus will be formed, is characterized by its lucid appearance and is lying above the main remnant of the macronucleus. This cell has a perfectly healthy appearance and should be contrasted with the animal shown in figure 17, which is an animal from Line III g, 202d generation, which several generations later died in the process of reorganization.

Paramaecium caudatum cells with one micronucleus and no macronucleus have been observed by Calkins, Kasanzeff and Hertwig. Calkins '04, fig. 14, pl. 2, illustrates such an animal. Abnormal animals of this character must not be confused with animals undergoing the reorganization process. The cell without a complete macronucleus (fig. 18), which we figure, is an entirely normal animal. Its sister cells carried on the race for nearly 70 generations more, some of them showing the crucial formation of macronuclear anlagen as will be described in detail in the next section.

C. Ascending phase. Before the onset of the ascending phase the same general cytological changes are to be observed as those we have described for Paramaecium aurelia, i.e., animals with only one micronucleus and a completely destroyed macronucleus, the remnants of which are scattered in the cell. This one micronucleus, as in Paramaecium aurelia, is the carrier of the life of the race through the new rhythm.

Maupas and Calkins in the conjugation of Paramaecium caudatum figure eight micronuclei, the products of the syncaryon, which are formed immediately after the separation of the conjugants. This we have verified in conjugation (fig. 25). In the reorganization process we have discovered animals at a slightly later stage with four micronuclei and four macronuclear anlagen, the latter representing four transformed micronuclei. The micronucleus which persists and carries the life of the race therefore must have undergone three divisions producing the four micronuclei and four macronuclear anlagen in the stage under discussion. The micronuclei (fig. 19) are characterized by their granular structure and somewhat glistening appearance. In the preparations the few granules appear pure blue without any reddish tinge. These micronuclei resemble, as far as one may judge from figures, one of the four micronuclei which Klitzke ('14, text fig. C, copied in the present paper as text fig. 5) shows in the conjugation of Paramaecium caudatum. This micronucleus (Klitzke's 'Micronucleusanlage,' d.k. 3) he believes undergoes degeneration together with two other micronuclei (d.k. 1, d.k. 2). The marked differences he points out are not observable in our specimen in the reorganization process, but this may be due to the fact that the animal has not yet reached this point. Or it may be that no micronuclear degeneration



Text fig. 5

occurs, as is the case in the description of this phase in the conjugation of Paramaecium caudatum as worked out by Calkins and Cull ('07, p. 396). We summarized in our former paper ('14, III, pp. 453, 454) the various opinions in regard to the formation of the macronuclear anlagen in Paramaecium, and showed that the problem is not settled since Maupas and Klitzke believe that four micronuclei and four macronuclear anlagen arise, three of the micronuclei undergoing degeneration. Maupas states definitely that four typical micronuclei are transformed into macronuclear anlagen. In the animal in the process of reorganization under discussion (fig. 19) this transformation of micronuclei into macronuclear anlagen has been completed. The anlagen are quite homogeneous, with the exception of a few granules frequently dispersed in a circle. In preparations stained with Delafield the anlagen show a diagnostic reddish tinge which cannot be expressed without colored plates. (Compare our present plates with those reproduced in colors in our paper on Paramaecium aurelia). The chromatin bodies are scattered throughout the cell in various stages of disintegration.

The next two figures (20 and 21) show only two micronuclei and four macronuclear anlagen in each animal. This stage may be interpreted as an animal in which two micronuclei have degenerated, or as an animal after the degeneration of three micronuclei and after the first somatic micronuclear division. In our work we found it necessary to study the cytology of conjugation of our pedigreed races of Paramaecium *caudatum* with particular reference to the fate of the four micronuclei which do not form macronuclear anlagen. In our races it appears clear that all four of the micronuclei do not persist and become distributed by the following two cell divisions as the definitive micronuclei of the four completely reconstructed Paramaecium cells. Figure 26 shows an exconjugant with clearly four macronuclear anlagen and four micronuclei. Figure 27 gives a pedigreed animal, after the first cell division subsequent to conjugation, in which only one micronculeus is present. Figure 28 shows a pedigreed animal, after the second cell division subsequent to conjugation, with a macronucleus and micronucleus.

This is in harmony with the stages in the reorganization process in our races having four anlagen and two micronuclei (figs. 20 and 21) which follow without a cell division the stage with four macronuclear anlagen and four micronuclei (fig. 19).

The reconstruction in the reorganization process of the typical vegetative Paramaecium cell is now effected by two cells divisions. Figure 22 shows an animal which has undergone both of these divisions. The cell has a single well developed anlage which has attained more typical macronuclear characteristics. The chromatin bodies are rapidly degenerating. The reorganized cell at the very beginning of the new rhythmical period is shown in figure 23.

A critical survey of the ascending phase shows that even in our small tube cultures, which might be compared with Kasanzeff's culture methods (except that he starved his animals while we attempted to supply ideal conditions), relatively few Paramaecium caudatum were able to accomplish the reorganization process. This is in agreement with Popoff's results because he found, according to our interpretation, only the early stages of the reorganization process. Calkins, though studying carefully his pedigreed cells, figures but one animal which we would interpret as showing the completion of the reorganization process ('14, III, text figs. 19 and 20), Hertwig has never observed what he considered a reorganization process in Paramaecium caudatum ('14, pp. 568, 569). We ourselves, using the same methods which proved successful in Paramaecium aurelia and also new ones adapted to the peculiarities of Paramaecium caudatum in culture, could not work out in such detail the uninterrupted sequence of endomictic events from pedigreed series of cells as in the case of Paramaecium aurelia. Nevertheless we have proved that the process of reorganization can be successfully accomplished in certain cases under pedigreed slide conditions though it is clear that these artificial slide cultures afford obstacles which the average caudatum cell finds it difficult to overcome when in the critical climax of the reorganization process or of conjugation. We believe the data presented establish beyond doubt that the reorganization process is a normal periodic event in the life history of Paramaecium caudatum.

IV. COMPARISON OF THE CYTOLOGICAL PHENOMENA OF THE REORGANIZATION PROCESS IN PARAMAECIUM CAUDATUM AND PARAMAECIUM AURELIA

This short outline of the cytological changes of the reorganization process in Paramaecium caudatum makes it clear, we believe, that there is no fundamental difference between the morphological features of this process in Paramaecium caudatum and Paramaecium aurelia, further than that incidental to the fact that the former species has one micronucleus and the latter two micronuclei in its typical vegetative stages.[3] The destruction of the old macronucleus and the formation of a new macronuclear apparatus of micronuclear origin is effected in both species.

However, there are some interesting minor variations which it may be well to contrast. The 'reduction' division in the reorganization process of Paramaecium caudatum with its 'dumb-bell' formation resembles more closely the phenomenon in the conjugation of this species than do the features of the 'reduction' micronuclear phenomena in the reorganization process of Paramaecium aurelia resembles those of conjugation in that species. We were unable to discover the same features in the 'reduction' division in Paramaecium aurelia during the reorganization process that were described by Hertwig for the comparable stages in conjugation. Hertwig ('14) figures a stage (text fig. 2, animal 3) which he interprets, together with the condition in the two previous animals (animals 1 and 2), as "Depressionserscheinungen von Paramaecium aurelia." We would interpret Hertwig's animal 3, on the basis of the animals figured in our earlier paper (fig. 12, pl. 1, etc.) as a cell in a stage of the reorganization process after the formation of four 'reduction' micronuclei two of which are already preparing for the second 'reduction' division. Animals with this morphological structure will actually complete the reorganization process, as we have proved ('14, III), and therefore such animals as figured by Hertwig cannot be interpreted as depression stages. Thus Hertwig indirectly and we directly prove that the 'reduction' micronuclei in Paramaecium aurelia in the reorganization process have somewhat different morphological characteristics from those of the same species in conjugation.

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[3] For a discussion of the specific characters of Paramaecium aurelia and Paramaecium caudatum and the literature on the subject, cf. Woodruff, Journal of Morphology, vol. 22, p. 223, 1911.

The destruction of the macronucleus before the formation of macronuclear anlagen in Paramaecium aurelia occurs, according to our observations, only by the extrusion of chromatin bodies from the macronucleus. But in Paramaecium caudatum there are clearly two methods of macronuclear dissolution in the descending phase of the reorganization process. One of these involves the breaking up of the macronucleus into one or more large pieces which finally degenerate in the cell (figs. 8, 9 and 10); the other involves the extrusion of chromatin bodies from the more or less intact macronuclear membrane (figs. 14, 15 and 16). This would seem to indicate that there may be also two distinct methods of accomplishing the ascending phase of the reorganization process in Paramaecium caudatum. One closely resembles the process in Paramaecium aurelia, being characterized by the presence of chromatin bodies, the absence of the old macronucleus in a cell with one micronucleus, this single micronucleus being the one which is to form the new nuclear apparatus. The other method is characterized by the breaking of the macronucleus into relatively large pieces and the streaming of chromatin out into the cytoplasm (figs. 10 and 13). Thus when the macronuclear destruction takes this form, there are few, if any, chromatin bodies to be found in the ascending phase. It may be mentioned incidentally that we have some data which suggest that under certain conditions merely a partial reorganization, not involving the formation of macronuclear anlagen, may lead, at least temporarily, to the continuance of the life of the line.

Our material did not allow us to prove whether the third so-called reduction division occurs or not, but we lean to the view, on the basis of our detailed study of this point in Paramaecium aurelia ('14, III, pp. 446–450 and p. 495) that this division actually is absent in the reorganization process in Paramaecium caudatum as in Paramaecium aurelia. Further, we have no indication of a cell division in the climax, and this point must be definitely settled for Paramaecium caudatum by further investigation.

Thus, although we have presented sufficient data to establish the occurrence of the reorganization process—endomixis—in Paramaecium caudatum, we have found no new fundamental facts to modify our brief theoretical suggestions as given in our earlier study of Paramaecium aurelia. We believe the suggestions—as there stated—must stand or fall on the basis of further study of endomictic phenomena in other Protista.

V. DISCUSSION AND CONCLUSIONS

We proved in our previous paper that a periodic reorganization process, to which we gave the name endomixis, occurred periodically throughout the seven years of the life of the main culture of Paramaecium aurelia. We showed in subcultures, from this main culture, in which conjugation was allowed to occur that lines derived from exconjugants underwent endomixis at the regular intervals. We thus proved that endomixis and conjugation are phenomena common to the same race of Paramaecium aurelia.

We showed further that endomixis occurred in a race of Paramaecium aurelia (culture B of our former paper) isolated in Germany. On the basis of this we stated ('14, II, p. 494, and '14, III, p. 474): "Therefore, the data justify the conclusion that this reorganization process is a normal phenomenon and probably occurs in all races of the species Paramaecium aurelia."

But since Hertwig intimates that endomixis is probably a peculiarity of Woodruff's main culture, we may cite further evidence to substantiate our former conclusion. We have had occasion, for certain experiments, to secure other races of Paramaecium aurelia. One of these was obtained from material sent to us by Prof. R. A. Budington of Oberlin, Ohio, and the other from material sent by Dr. Florence Peebles from Bryn Mawr, Pennsylvania. These two races, taken at random from material collected at widely separated localities, immediately showed endomixis at periods similar to those of the races already studied.

Thus endomixis has now been demonstrated in each of the four races which we have studied.

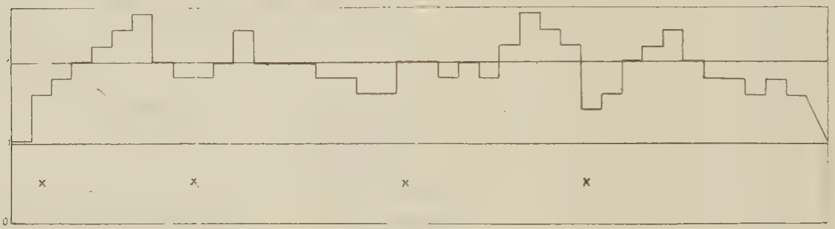
Further, the idea of Hertwig that endomixis occurs only after long cultivation of a race of Paramaecium was shown not to be true in Woodruff's main culture. We stated ('14, II, p. 492) that animals preserved during the first year of its cultivation showed stages of the process, and further we stated that the race from Berlin showed stages of endomixis very early in its history ('14, II, p. 493). Now, with this point in mind, we have found endomixis in each of the new aurelia races within the first thirty days of their life in culture.

Therefore, we have proved that endomixis is a phenomenon common to all four races of Paramaecium aurelia which we have studied and thus it is highly probable that it occurs in all races of this species. Further, we have proved that endomixis is not a phenomenon which is gradually acquired after long pedigreed culture but is completely developed in animals at the time of isolation from wild cultures, and still further we have proved that endomixis is a potentiality of lines which have the power of conjugation ('14, III, p. 473).

Having, we believe, disposed of these questions which have been raisedin regard to our work on Paramaecium aurelia, we are in a position to return to endomixis in Paramaecium caudatum. The cytological phenomena of this process have been presented in the previous sections, and we believe that we established beyond peradventure the truth of our statement ('14, III, p. 475) that endomixis "occurs at least with essentially similar features in Paramaecium caudatum also" and therefore that endomixis is a regular normal periodic process in the life of Paramaecium caudatum (text fig. 6).

Early work on Woodruff's main culture of Paramaecium aurelia ('09, '11) showed that there are periodic fluctuations (rhythms) in the rate of reproduction which are not the results of environmental variation, but which are due to some periodic internal phenomena of unknown character (Woodruff and Baitsell, '11). We have shown ('14) that endomixis is the underlying internal process whose physiological effect had been observed but whose nature had only been suspected ('14, III, p. 430), and stated ('14, III, p. 481) "Therefore, it is evident not only that the reorganization process is coincident with the low points between two rhythms, but also that there is a causal relation between the reorganization process and the rhythms."

We have now established this same conclusion for Paramaecium caudatum. In our races of this species, however, the endomictic periods appear at intervals of about 50 to 60 days, or from 80 to 100 generations, instead of from 25 to 30 days, or 40 to 50 generations as in Paramaecium aurelia. But they are fundamentally the same morphologically and physiologically in both species.



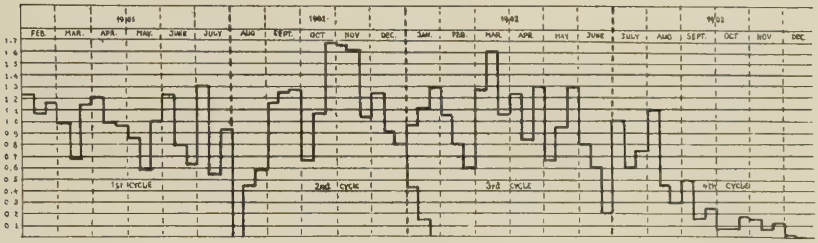
Text fig. 6 Graph of the rate of division of Culture Z, Line I, averaged for five-day periods. The periods during which the reorganization occurred are indicated by a ×. Cf. page 71, table 3; and also Woodruff and Erdmann, '14, III, text figures 16 and 17.

Now a critical examination, in the light of our present knowledge of endomixis, of the division rate of Calkins' pedigreed culture of Paramaecium caudatum, the study of which led him to his well known conception of protoplasmic old age in Protozoa, shows clearly that his periods of degeneration can be perfectly interpreted as endomixis (text fig. 7, copied from Calkins). Woodruff in an early paper ('09, p. 300) wrote:

> I have previously interpreted as rhythms the tri-monthly depressions in vitality, which Calkins and earlier workers on Paramaecium have noted, and the results obtained from my culture of Paramaecium seem to indicate that the semi-annual cycles of Calkins are also actually rhythms, recovery from which was not autonomous under the conditions of a constant environment. The general occurrence of rhythms in the life history of infusoria is established, I believe, but to what they are due is still awaiting discovery.

In other words the present studies on Paramaecium caudatum show that most if not all the depression periods of Calkins are undoubtedly rhythms and the 'cycle' is non-existent—it is merely, as stated above, a rhythm at which the organisms are unable to recover autonomously by endomixis owing to the more or less artificial culture methods imposed in daily isolation pedigreed cultivation (text fig. 7, copied from Calkins, '04).

It may be well, in view of the recent comments by Hertwig ('14) and by Calkins ('15) in their discussion of our paper on endomixis, to state the position of the problem of depression and the significance of conjugation in Paramaecium in 1907 when Woodruff began his work on Paramaecium.



Text fig. 7 "History of the A Series from start (Feb. 1, 1901) to finish (Dec. 19, 1902) by ten-day periods (three periods to each month). The ordinates represent the average daily rate of division. The heavy dotted lines indicate the limits of the several cycles, and the lines of the curve carried to the base indicate that the individuals that were not stimulated by change of diet died out. The points at which such lines leave the curve indicate the time of the successfully changed diet." (Calkins, '04, p. 426.)

The consensus of opinion of biologists, chiefly on the basis of the work of Maupas, Calkins and Hertwig, was that infusoria are able to reproduce by division for only a limited number of generations, after which protoplasmic old age, depression, and physiological death ensue. For this the sole panacea was conjugation. But Woodruff found that by supplying proper environmental conditions it was possible to breed a pedigreed race of Paramaecium aurelia indefinitely (so far, April 1915, more than 5000 generations) without recourse to conjugation. Therefore, he concluded, in direct opposition to Maupas, Calkins and Hertwig, that conjugation is unnecessary for the indefinite life of Paramaecium under favorable environmental conditions.

To Hertwig's and Calkins' recent contentions that their conclusion was correct and Woodruff's was wrong, we would reply, that the reorganization process (endomixis) is not conjugation and no one had any other phenomenon than conjugation—involving syncaryon formation—in mind until Woodruff and Erdmann discovered endomixis—in which a syncaryon is not formed. To say that endomixis fills essentially the same rôle in the life history of the infusorian as conjugation is to beg the entire question. Had Hertwig worked out the stages which he found over twenty-five years ago, or realized their general significance, and had he related these with rhythms ten years ago when they were discovered, the problem would have been cleared up then. The aspect of the problem has changed with the discovery of endomixis. The question is now not whether conjugation is necessary—for Woodruff has shown that it is not—but whether endomixis, a new phenomenon which Woodruff and Erdmann have shown to exist, is necessary.

Whatever the answer afforded by future work to this new question may be, it is clear that conjugation—and by this always was meant, and always is meant, the formation of a syncaryon—is not necessary, since an individual Paramaecium is self-sufficient to reproduce indefinitely without recourse to conjugation.

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VII. EXPLANATION OF PLATES

All the figures represent specimens of Paramaecium caudatum. The animals were fixed in Schaudinn's sublimat-alcohol and stained with Delafield's hematoxylin. The drawings were made from total preparations, with Abbe camera lucida, Zeiss homogeneous immersion 2 mm. and compensating ocular 12, with drawing board level with stage of microscope. Magnification, about 1500 diameters. Reduction in reproducing plates 1, 2, 3, 4, 5 is one third; plates 6 and 7 is one half.

PLATE 1

EXPLANATION OF FIGURES

1 Culture Y, Line IV, 85th generation, April 3, 1914. Normal animal in a period when endomixis is not in progress.

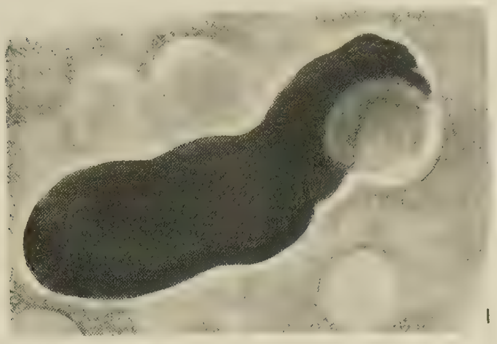
2 Culture Y, Line IV ae, 136th generation, April 28, 1914. Typical animal just at the start of endomixis.

3 Culture Y, Line III fa, 203d generation, June 8, 1914. Animal just in a very early stage of endomixis. Macronucleus wrinkled. Micronucleus has shifted from its typical position close to the macronucleus.

4 Culture Y, Line III fb, 203d generation, June 8, 1914. Animal in a slightly more advanced stage than the one shown in figure 3.

5 Culture Y, small tube culture, about 137th generation, April 22, 1914. Start of the first 'reduction' division.

6 Culture Z, Line IV, 184th generation, December 24, 1914. Macronucleus partly devoid of chromatin. First 'reduction' division nearly completed.



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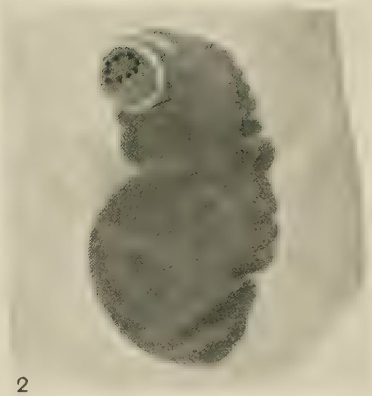

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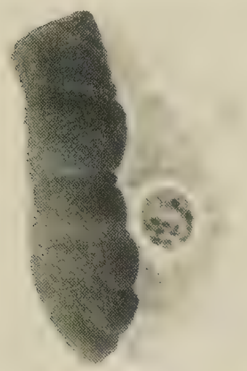

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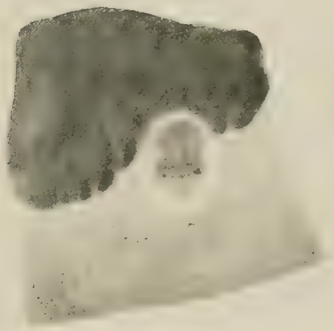

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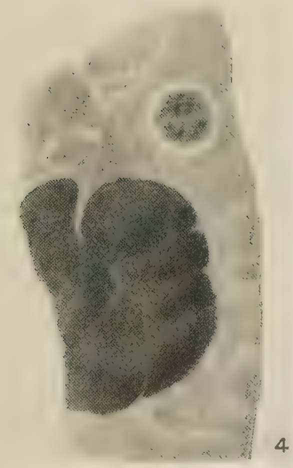

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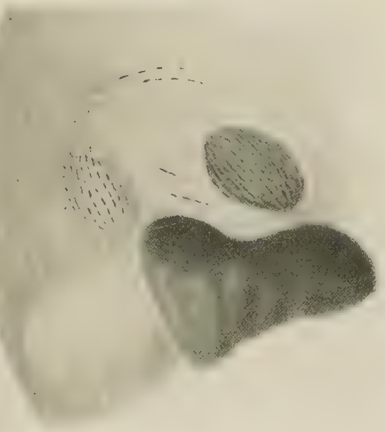

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PLATE 2

EXPLANATION OF FIGURES

7 Culture Y, Line III g, 202d generation, June 7, 1914. Markedly wrinkled macronucleus and one chromatin body. Part of crescentic stage of micronucleus shown protruding from under the macronucleus.

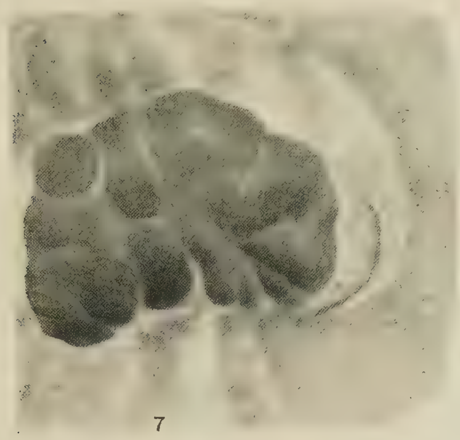
8 Culture Y, small tube culture, about the 82d generation, April 1, 1914. Macronucleus broken into several large pieces. Elongated micronucleus, one pole partly devoid of chromatin.

9 Culture M, Line II, 89th generation, February 19, 1915. Completed fragmentation of macronucleus into two parts.

10 Culture Y, small tube culture, about the 137th generation, April 22, 1914. Animal with macronucleus breaking into two parts. First 'reduction' micronuclear division completed.

11 Culture M, Line I, 90th generation, February 19, 1915. Macronucleus broken into two pieces from which chromatin is streaming and forming chromatin bodies. Two micronuclei are present, one of which is drawn. The presence of numerous food vacuoles is due to transferrence of this animal to rich hay infusion medium previous to killing.

12 Culture Y, Line IV, 26th generation, March 2, 1914. Two 'reduction' micronuclei are present.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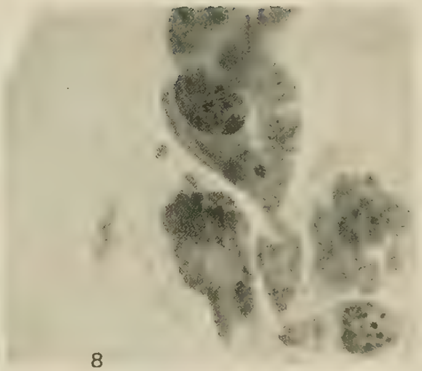
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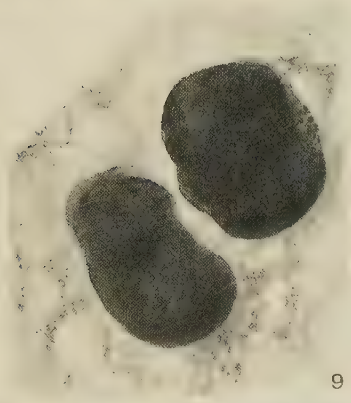
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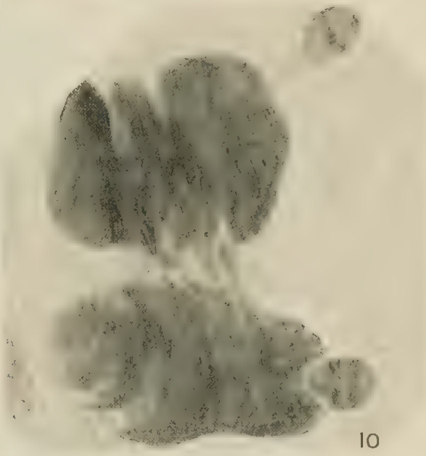
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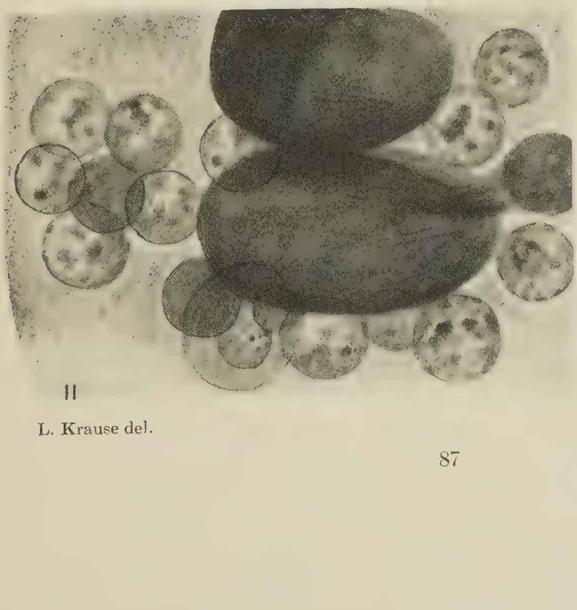
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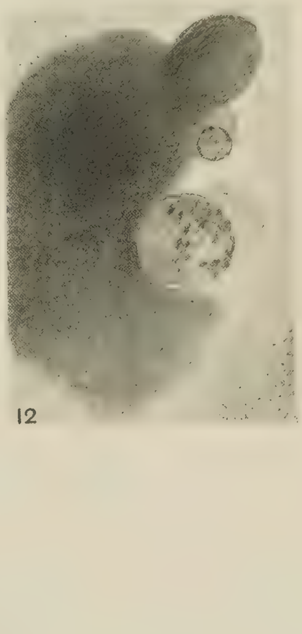
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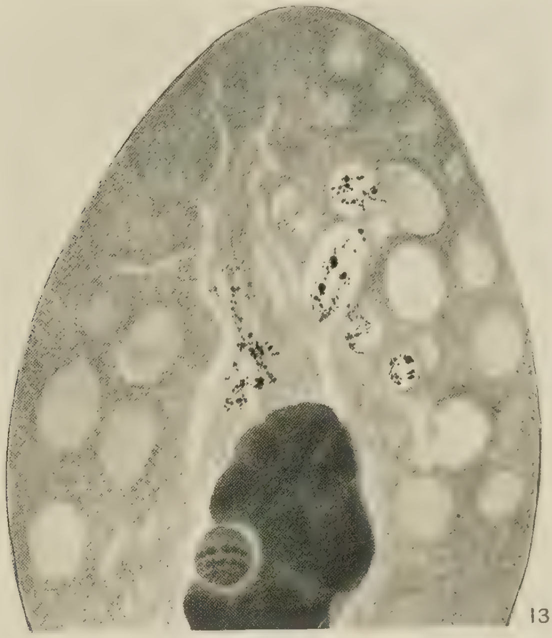
PLATE 3

EXPLANATION OF FIGURES

13 Culture Z, Line I, 91st generation, November 5, 1914. Illustrating the method of macronuclear dissolution by streaming out of chromatin. Micronucleus shows the characteristic band formation of early endomixis.

14 Culture Z, Line I, 93d generation, November 6, 1914. Macronuclear dissolution by elimination of chromatin bodies. Single micronucleus close to macronucleus. Five food vacuoles shown in the cell.



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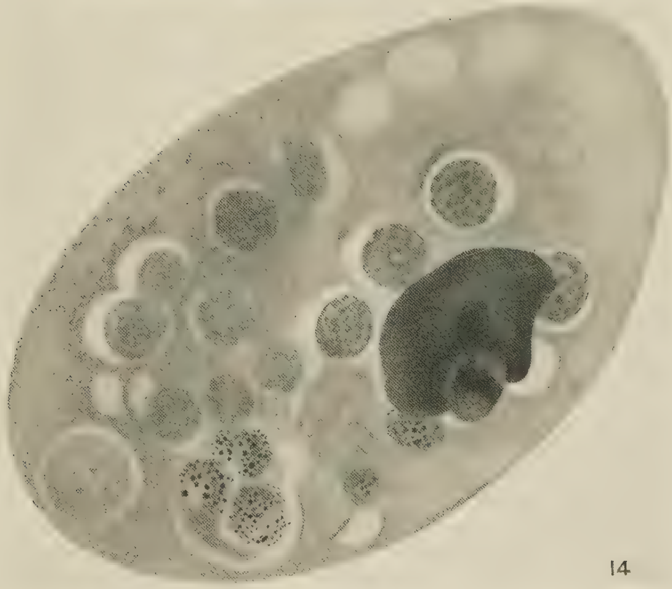

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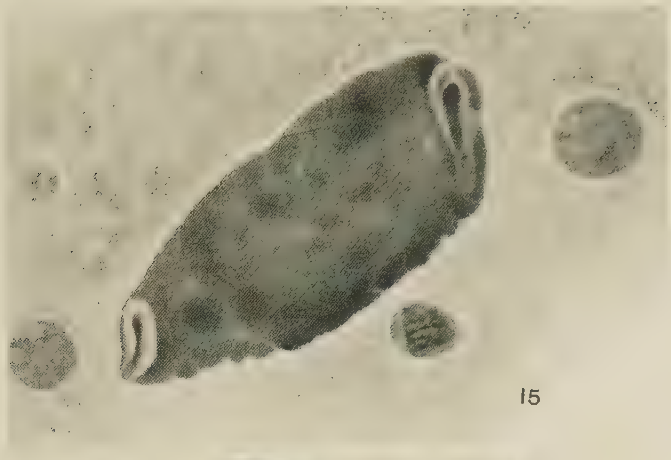
PLATE 4

EXPLANATION OF FIGURES

15 Culture Y, Line IV, 18th generation, February 26, 1914. Descending phase. One micronucleus and some chromatin bodies visible.

16 Culture Y, small tube culture, about 137th generation, April 22, 1914. Descending phase. One chromatin body is in the macronucleus, others have left it.



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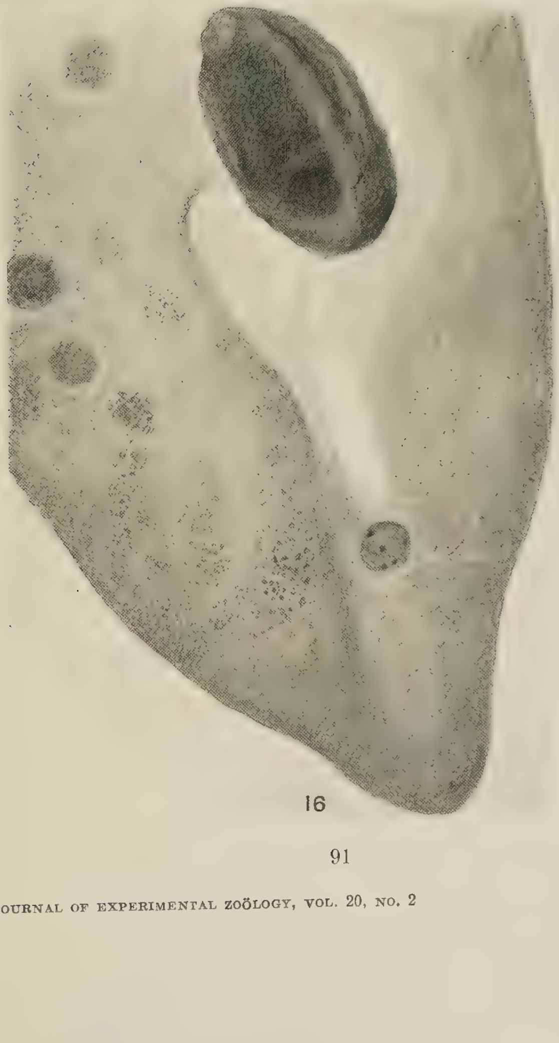

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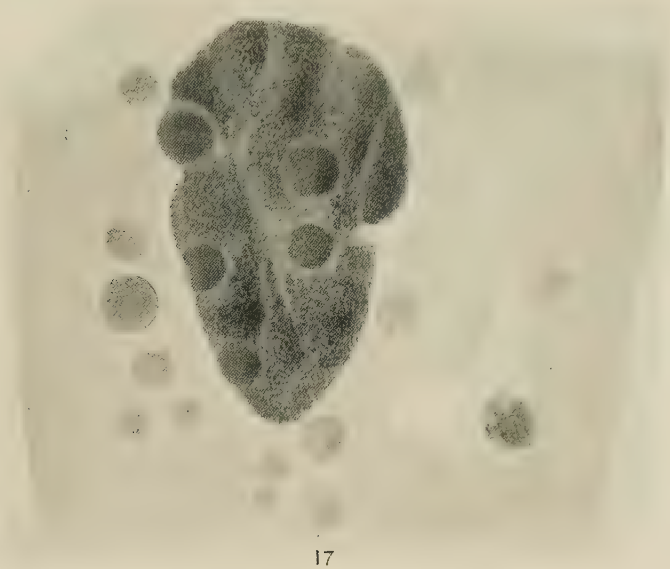
PLATE 5

EXPLANATION OF FIGURES

17 Culture Y, Line III g, 202d generation, June 6, 1914. To illustrate that essentially the same stage as that shown in figure 14 from Culture Z occurred in another Culture Y in isolated animals.

18 Culture Y, small tube culture, about 137th generation, April 22, 1914. Animals in the climax of endomixis. Macronucleus partially resolved into degenerating chromatin bodies. Micronucleus after 'reduction.'



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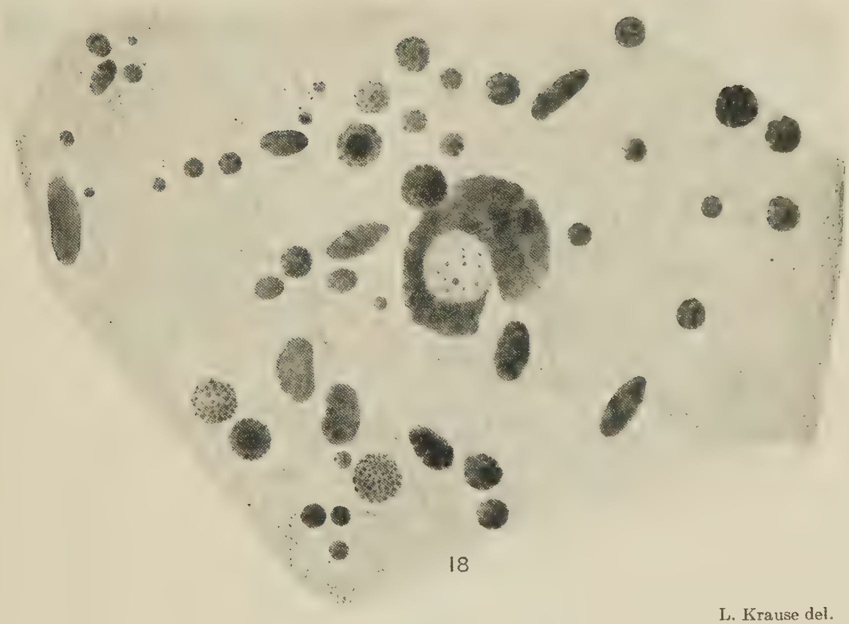

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PLATE 6

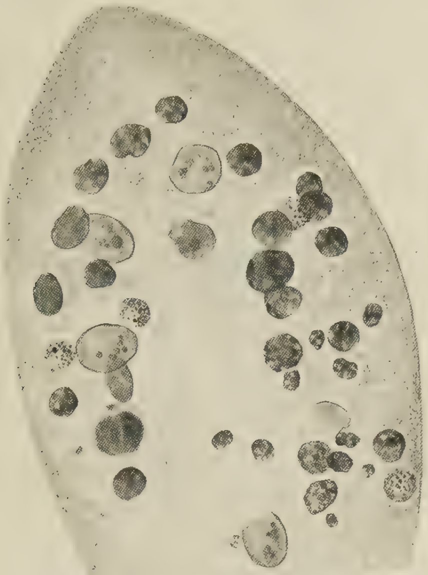
EXPLANATION OF FIGURES

19 Culture Y, small tube culture, about 137th generation, April 22, 1914 Animal in ascending phase of endomixis. Four micronuclei and four macronuclear anlagen present. Numerous chromatin bodies in all stages of degeneration.

20 and 21 Culture Y, small tube culture, about 82d generation, April 1, 1914. Animals in ascending phase. Two micronuclei and four anlagen are present.

22 Culture Y, small tube culture, about 82d generation, April 1, 1914. Animal in the ascending phase, showing one anlage.

23 Culture Y, small tube culture, about 82d generation, April 1, 1914. Paramaecium cell immediately after undergoing endomixis.



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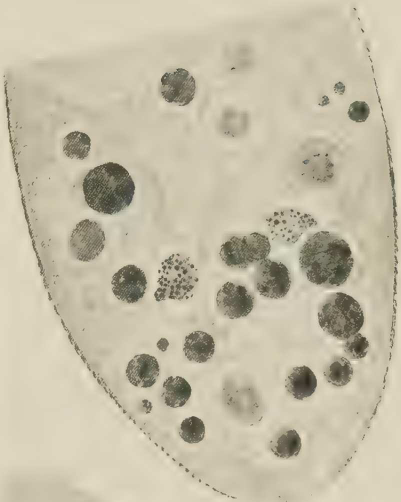

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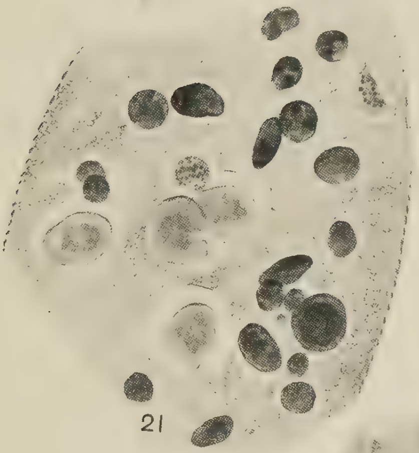

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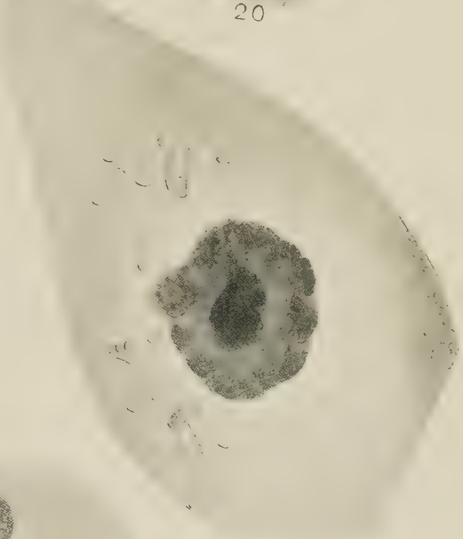

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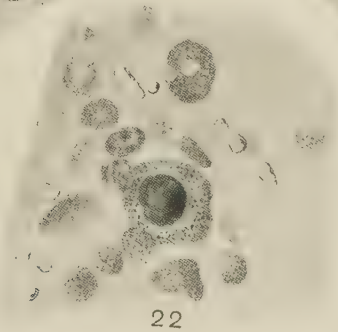

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PLATE 7

EXPLANATION OF FIGURES

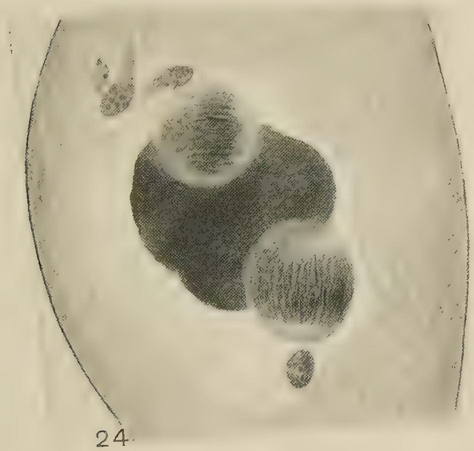
24 Culture Z, March 27, 1915. Exconjugant. Division of syncaryon.

25 Culture Z, March 27, 1915. Exconjugant. Macronucleus show typical ribbon formation. Micronuclear divisions preliminary to formation of macronuclear anlagen.

26 Culture Z, March 27, 1915. Exconjugant. Four macronuclear anlagen and four micronuclei.

27 Culture Z, April 4, 1915. Individual from the first division of exconjugant.

28 Culture Z, April 5, 1915. Individual from the second division after conjugation.



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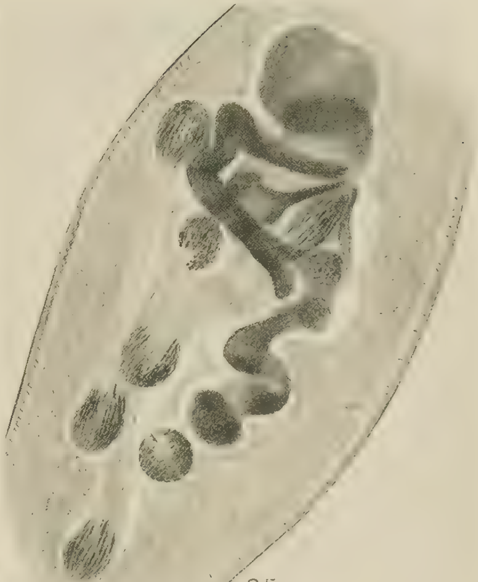

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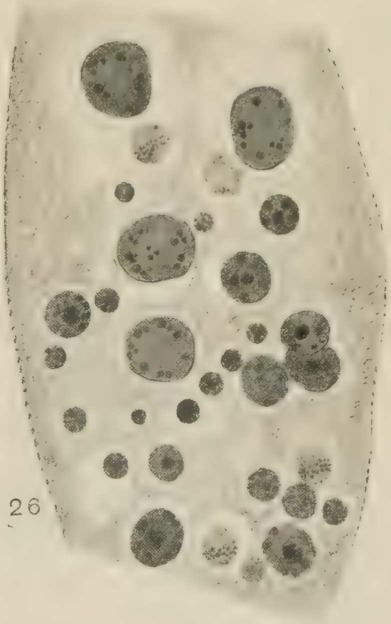

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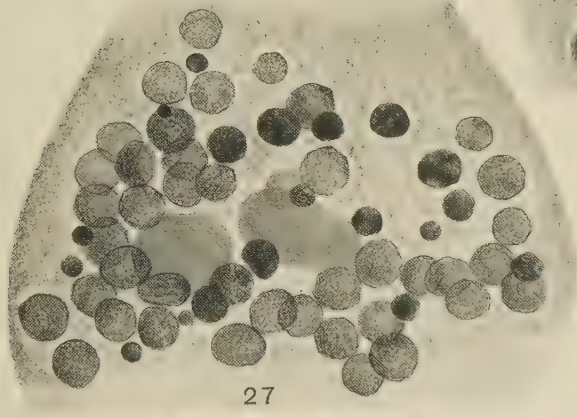

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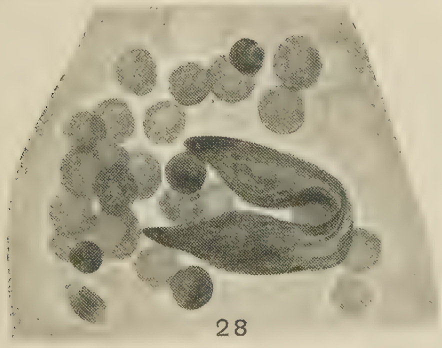

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[*Reprinted from* SCIENCE, *N. S., Vol. XLIII., No. 1104, Pages 263–270, February 25, 1916*]

THEODOR BOVERI[1]

WITHIN a single year after Weismann's death our science has suffered another severe blow in the loss of Theodor Boveri, who died in Wuerzburg on October 15 at the age of fifty-three years. Pioneer and leader in the fields of cytology and experimental zoology, his loss will be felt keenly in this country where he had so many friends and pupils and where his field of research has been so popular during the past two decades. Boveri's personal life was very simple, always devoted to his work, his family and the pleasure coming from a deep love for art and nature. A native of Bavaria, he studied first philosophy and later zoology in Munich. His doctor's thesis on the structure of the nerve fibers in vertebrates treated a subject to which he did not later return. For, encouraged by his teacher, Richard Hertwig, soon after receiving his degree he entered the field of cytological research. Here, following the example of his teacher, he combined practically from the beginning the morphological and experimental methods.

His very first work in this line proved to be a great success, securing to him the *venia legendi* as privat dozent in the University of Munich. A few years later, when only thirty years of age, he was called to Wuerzburg, to succeed Semper in the chair of zoology and comparative anatomy. Here he remained during the rest of his life with the exception of frequent trips to the zoological stations of southern Europe, especially Naples, where he was a regular guest. He also made a short visit to the United States. His reputation as an investigator soon attracted scores of students to the quiet laboratory in Wuerzburg, many of them coming from this country. One of the first, Miss O'Grady, of Vassar College, became his faithful wife, the mother of his daughter and an efficient assistant in all his later scientific work.

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[1] Paper read before the Biological Club, Yale University, December 3, 1915. I am greatly indebted to Professor Wesley R. Coe for kindly revising the manuscript.

It is hardly necessary to add that with his growing fame came numerous honors conferred on him by his university, where he held the highest office as rector magnificus in 1909, by his government and by learned societies. Among the learned societies which conferred on him their membership is the American National Academy of Sciences.

When Weismann resigned his professorship in Freiburg Boveri was called to succeed him, but declined. Later the directorship of the new research laboratory of the Kaiser-Wilhelm-Gesellschaft in Berlin was offered to him. He first accepted, worked out the whole organization and brought together the staff; but suddenly he declined again. Possibly he already felt that his health was no longer vigorous enough for such a change. When I saw him for the last time, two years ago, in Naples, he gave me the impression of a strong and healthy man, but within a short time a disease of the gall-bladder forced him to interrupt his teaching for a year. An operation performed in the first days of October could not save his life.

When Boveri entered the field of biological research in the middle of the eighties the science of cytology was just outgrowing its childhood. Only ten years previously the fundaments had been laid. After certain incidental observations, especially by A. Schneider and Auerbach, Otto Buetschli collected his results on the division of the cell, the maturation and fertilization of the egg and the conjugation of Infusoria in his classic work of 1876. From that time dates the knowledge of the karyokinetic division of the cell with all its consequences. At about the same time appeared O. Hertwig's classic work on the fertilization of the sea-urchin egg, making it clear for the first time that fertilization is the union of egg- and sperm-nucleus. Then followed one fundamental discovery after another. Strasburger soon applied the new facts to the cells of plants. Flemming (1882) worked out the details of the mitotic figure, introduced the term "chromatin" and discovered the longitudinal splitting of the chromosomes. Roux (1883) realized the theoretical importance of the new discoveries and pointed out the meaning of the mitotic division of the cell, anticipating practically all of the views of to-day. In 1884 Heuser for plants and Van Beneden for animals were able to prove that the separated halves of the chromosomes are distributed to the daughter cells. (The word chromosome was first introduced in 1888 by Waldeyer.) At the same time Naegeli (1884) developed his ingenious theory of the idioplasm, and soon Strasburger, Koelliker, O. Hertwig, Weismann pointed to the chromosomes as the seat of the material basis of heredity. Only one important step was still lacking, the full understanding of the process of fertilization. Mark (1881) came very near to making this discovery, but it was Van Beneden (1884) who proved that in fertilization the same number of paternal and maternal chromosomes are handed over to the cleavage cells. These discoveries were made on the eggs of *Ascaris*, studied previously by A. Schneider and Nusbaum, and which have since become one of the classic objects of cytology. One after the other followed in those days the discoveries, which elucidated the whole process; the meaning of the polar bodies (Buetschli, O. Hertwig, Giard, Mark); the parallelism between ovogenesis and spermatogenesis (Van Beneden et Julin); the theory of the reduction division (Weismann); the behavior of the polar bodies in parthenogenesis (Blochmann, Weismann and Tshikawa); the continuity of the germ-plasm (Nusbaum, Weismann); the individuality of the chromosomes (Rabl); and finally, in 1887, the foundation of experimental cytology by O. and R. Hertwig. This was the year when Boveri's first "Zellstudien" appeared.

Under the influence of Van Beneden's classic book, Boveri began by studying the sex cells of *Ascaris*. In his Zellstudien, I., 1887, he takes up the subject of the formation of the polar bodies. In harmony with Buetschli's discovery Schneider and Nusbaum had described the formation of the polar bodies in *Ascaris* as a regular mitosis, whereas Van Beneden and Carnoy insisted that it was a different process. Boveri proved that the former view is correct and was able to explain many discrepancies of these authors by discovering that there are two different varieties of *Ascaris* in regard to their number of chromosomes, called to-day univalens and bivalens. It is of interest to note that he expresses here the view that the recently discovered formation of a single polar body in parthenogenetic eggs may be explained by the assumption of a fertilization of the egg nucleus through the second polar nucleus. In 1888 appeared Boveri's Zellstudien II., dealing with the fertilization and division of the *Ascaris* egg. Here we find—besides many morphological details—his formulation of the theory of the individuality of the chromosomes, founded, as he freely recognized, by Rabl, and since one of the fundamental principles of cytological research. And he furnished important proofs by comparing the prophases of the division with the last telophases, and further by showing, that in cases of abnormal distribution of the chromosomes as many of them came out of the resting nucleus as had entered it. He was further especially interested in the mechanics of cell-division. He attributed a great importance to the special plasm surrounding the centrosome, the archoplasm (a theory abandoned later by him), and pointed to the importance of the continuity of the central bodies called by him centrosomes, already discovered by Van Beneden. And here we find developed also his idea, that the main importance of fertilization is the introduction of a centrosome into the egg. Starting from some abnormal cases, where a division of the cell is possible without a nucleus, he reached the conclusion that the centrosome is the dividing-organ of the cell. It is of importance to note that he emphasized even in this early paper (pp. 10–11) the necessity of experimental analysis of the phenomena of fertilization and heredity, recently inaugurated by the brothers O. and R. Hertwig.

To all these problems studied in *Ascaris* he furnishes a supplement in Zellstudien III., 1890, by applying the same studies on many marine invertebrates during a sojourn at Naples. For each of the objects investigated he could prove Van Beneden's law concerning the chromosomes in fertilization to be correct. Further he shows that in all these animals the reduced number of chromosomes is found even at the beginning of the maturation divisions in both sexes. The real reduction, therefore, must occur as early as in the oogonia and spermatogonia. It may be added here that during these years the complete parallelism of the cycle of male and female sex cells was definitely proven by the work of Van Beneden et Julin, Boveri, Platner and O. Hertwig, and that at the same time the problem of the reduction division was solved through Henking's idea of a conjugation of the chromosomes (the term introduced by Boveri), proved to be true by Rueckert (1891). As the final word of all his studies during these years may be regarded his article "Befruchtung" in Merkel und Bonnet's Jahresbericht, 1891, where he reviews the whole field in his keen and masterly way. It is of special importance that here were published the first figures of the process of diminution of the chromosomes, some years previously discovered by him in the cleavage cells of *Ascaris* and fully understood in its importance for the doctrine of the Keimplasma.

It has been stated already how keenly Boveri felt the necessity of applying experimental methods to the study of cytology. His first papers in this direction were published in 1888 and 1889. The latter especially gave a great impetus to our science, his famous report, "Ueber einen geschlechtlich erzeugten Organismus ohne muetterliche Eigenschaften."

The brothers Hertwig had succeeded in rearing fragmented eggs of Echinoderms up to the gastrula stage and had been able to fertilize enucleated fragments of sea-urchin eggs. Boveri conceived the very ingenious idea of using this method, to determine whether or not the hereditary qualities are transmitted in the nucleus. He therefore fertilized enucleated egg-fragments of *Sphærechinus* with the sperm of *Echinus* and raised the resulting larvæ to the pluteus stage. He believed he was able to prove that these larvæ exhibited only paternal characters. It is well known that the validity of this conclusion was attacked by Morgan and by Seeliger. It was not until 1896 that Boveri published, in *Roux's Archiv,* the full account of this work and answered the objections of his critics. To-day we know from the work of many observers that the question is not a simple one. But in this paper we find incidentally another discovery, taken up by Boveri much later; namely, the dependence of the size of the larval nuclei upon the number of their chromosomes.

It is well known that during this first decade of Boveri's work our science was revolutionized. In the years 1884–88, Wilhelm Roux had laid the foundations of the science of Entwicklungsmechanik and the brothers Hertwig had started their experimental work in cytology and hybridization. Soon Driesch (1891) imbued the new science with his philosophical spirit, while J. Loeb (1891) attacked similar problems from a physiological point of view. Soon Morgan, Wilson and Herbst joined these pioneers and this line of work henceforth made itself felt also in all of Boveri's.

After some smaller papers, dealing with experiments relating to the theory of mitosis, he published in 1899 a full account of the facts relating to the diminution of the chromosomes, long since discovered by him.[2] To make all the facts clear he had to give a full account of the cell-lineage of this worm, a line of work of the greatest importance since the discoveries of Wilson and Conklin in the early nineties (although the foundations of this line of work date back to the investigations of Rabl, Van Beneden and Whitman, as is well known). The facts were in harmony with the results of Zur Strassen, which had in the meanwhile been published.

The year 1900 brought the fourth part of the Zellstudien, with the subtitle "Ueber die Natur der Centrosomen." The thirteen years which had passed since the publication of the first fascicle had seen an immense accumulation of morphological and physiological facts regarding the various parts of the cell, especially the chromosomes and the centrosomes. The importance of these latter for the mechanism of cell-division was already recognized by Buetschli as early as 1876, in spite of the fact that he did not realize them as distinct bodies. Flemming made this discovery, the significance of which was realized, however, only when Van Beneden and Boveri had discovered the life cycle of these bodies and recognized them as permanent organs of the cell, and after Boveri had pointed to their important bearing on the theory of fertilization. Since that time a vast accumulation of knowledge concerning the centrosomes had been acquired through the work of Brauer, Coe, Griffin, Haecker, Heidenhain, Kostanecky, Lillie, MacFarland, Mead, Meves, Van der Stricht, Vejdovsky, Wilson and others. Boveri now deals with all the questions which had been raised, adding a series of new facts about the life cycle of the centrosomes in different objects. He discusses the question of the nuclear origin of the centrosome in the male sex cells of *Ascaris,* discovered by Brauer and confirmed by Boveri's pupil Fuerst. Then came the question of the persistency of the centrosome in non-dividing cells according to Heidenhain, and the centrosome theory of the basal bodies of ciliary cells as developed by Henneguy and Lenhossek. Great importance was attributed to the question regarding the phylogeny of the centrosomes, discussed at this time in connection with the discoveries in Protozoa by Buetschli, R. Hertwig, Blochmann, Schaudinn and Calkins. Further he deals with the rôle of the centrosome in the mechanism of cell-division, which had been discussed broadly from a physical standpoint during these years by Buetschli, Heidenhain, Rabl, Ziegler and Rhumbler, and defends his old earlier viewpoint. Then he refuses Fischer's destructive criticism of the methods of microscopical research; and finally tries to bring his views into accord with Morgan's discovery of the artificial astrospheres and Loeb's artificial parthenogenesis. Much space is devoted to the question concerning the relation of centrosome and centriole, a subject which is no longer considered of great importance. In connection with this paper may be mentioned his address before the Versammlung Deutscher Naturforscher und Aerzte 1901, "Das Problem der Befruchtung," where he again puts forward his centrosome theory of fertilization and endeavors to reconcile it with Wilson's new work upon the cytology of artificial parthenogenesis.

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[2] "Die Entwicklung von *Ascaris megalocephala* mit besonderer Ruecksicht auf die Kernverhaeltnisse," Festschr. f. C. von Kupffer, 1899.

In 1903 Boveri published a preliminary report of his work upon multipolar mitosis, which investigation is, in the writer's opinion, the acme of his cytological work. Fol and O. Hertwig had discovered the simultaneous division of dispermic sea-urchin eggs into four cells. Driesch had separated these four blastomeres and raised stereoblastulæ from them. Boveri now uses this method for attempting to analyze the different qualities of the chromosomes in one cell. He demonstrated that the four cells derived from a tetraster-division may get every possible combination of the 3 × 18 available chromosomes; and that the distribution of normality or deficiency in the plutei raised from the isolated cells corresponds exactly to the probable content of the cells in regard to a complete or incomplete set of the qualitatively different chromosomes. These facts are to-day so well known to every biologist that they do not need to be exposed further. But it might be said that the full account of the work published in 1908 as Zellstudien VI., shows Boveri's analytical genius from its very best side; the reading of this work is a highly intellectual and esthetical pleasure. There may be incidentally mentioned here a short paper on the influence of the sperm on the larval characters of Echinids.[3] This paper based on hybridization experiments proves, contrary to the views of Driesch, that all larval characters are influenced by the sperm cell.

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[3] Roux's Archiv, 16, 1903.

The same year Boveri reviews before the German Zoological Society the knowledge "Ueber die Constitution der chromatischen Kernsubstanz," a lecture that made a great impression on his hearers through his usual crystalline clearness and keen analysis. It is remarkable because he accepts here unreservedly the recently published hypothesis of McClung regarding the accessory chromosomes as sex-determiners; further, Suttons's analysis of the relation between the distribution of the chromosomes and Mendelian characters, a hypothesis which Boveri had conceived independently, but had not previously published, besides a brief remark pointing to his occupation with the subject. In this connection it might be said that it is characteristic of Boveri's work that important discoveries are mentioned in his papers occasionally, but not communicated *in extenso,* because he intended to work them out more fully later. So he always returns to his former observations after a great many years. Meanwhile there may have been done much work in the same line and ideas proposed from other sides, that he had himself in mind. And this often caused discussions about priority. So Boveri returned in 1905, in Zellstudien V., to his old discovery of 1889 that the size of nuclei in normal and merogonic larvæ of Echinids corresponds to the number of chromosomes they contain. The question of size relations between nucleus and cytoplasm had meanwhile become very important through the work of Gerassimoff (1902) and especially R. Hertwig (1903), who tried to base an analysis of many phenomena of cell-life on the assumption of a nuclear-plasmic relation. Boveri now had the ingenious idea of studying the relation between the number of chromosomes and nuclear and cell size by comparing the cells of Echinid larvæ experimentally produced with different chromosome numbers. There he had larvæ, called hemikaryotic, with the haploid number of chromosomes, obtained by artificial parthenogenesis (thelykaryotic) or by merogony (arrhenokaryotic); further, the normally fertilized, diploid or amphikaryotic larvæ, with the normal number of chromosomes, *i. e.*, twice as many as the foregoing, then diplokaryotic larvæ, again with twice as many chromosomes as the last, produced by artificial suppression of the first cleavage figure. Now by comparing these larvæ he found that the surface of the nuclei is proportional to the number of chromosomes contained in them; that the size of the cell is again proportional to both; and that the number of the cells in the same stage is inversely proportional. It does not need to be said that he discussed all consequences from these facts, in their different aspects. It is well known that these discussions are still going on, especially in connection with the work of R. Hertwig and his pupils and of Conklin.

The ever-growing tree of cytological research had meanwhile developed another flourishing branch. Henking had discovered (1891) the facts about the accessory chromosomes without understanding their importance. The studies of Montgomery and Sutton again revived in the beginning of the century the interest in these facts. McClung recognized in 1902 their importance for the sex-problem, and the work of Miss Stevens and especially Wilson brought the most surprising clearness. Boveri immediately became interested in these questions and suggested to some of his students lines of work in that direction. In 1909 he reported before the "Physikalisch-medizinische Gesellschaft" in Wuerzburg, where practically all his discoveries were first communicated, Miss Boring's work, discovering the very important *Ascaris* type of sex-chromosomes; further about von Baehr's work, who cleared up simultaneously with Morgan the interesting behavior of the sex-chromosomes in the male cells of aphids; further about Gulick's studies on the sex-chromosome cycle of Strongylids, especially important because he was the first to work out in detail the conception that sex-linked characters are carried by the x-chromosome; finally Baltzer's work about sex-chromosomes in female Echinids (which later had to be revoked after Tennant's work). Boveri himself studied the sex-chromosomes in hermaphroditism (1911) and succeeded, simultaneously with Schleip, in bringing the facts in harmony with the general conceptions; the object was the nematode *Rhabditis nigrovenosa,* which shows an alternation between hermaphroditic and bi-sexual generations.

The last years of Boveri's life gave to us three more papers in the general field of cytology, each one showing him still at the summit of his intellectual strength. The first, "Ueber die Charaktere von Echinidenbastardlarven bei verschiedenem Mengenverhaeltnis muetterlicher und vaeterlicher Substauzen" (1914), gives a very fine analysis of the relative importance of protoplasm and nucleus in the inheritance of characters. By comparing hybrid larvæ with different qualities of both (developed from giant-eggs, fragmented eggs, isolated blastomeres) he reaches the conclusion that the chromosomes are responsible for the characters of the larvæ (in agreement with Baltzer and Herbst and opposed to Godlevski). In the second paper, "Zur Frage der Entstehung maligner Tumoren" (1914) we find Boveri in a field at first sight far distant from his usual line of work. But only apparently. In his former analysis of the chromosomes in multipolar spindles he had already pointed to the possibility of explaining the sudden origin of malignant tumors and their behavior by the assumption that they originate from cells with abnormal combinations of chromosomes resulting from an occasional multipolar mitosis produced by some influence in the surrounding medium. As he believes that this idea, very closely connected to von Hansemann's cancer-theory, might be useful for further research, he works it out here in extenso and discusses its merits in regard to the facts of pathology. The third paper finally, and the last one published by Boveri during the summer 1915, deals again with a subject, discussed by him 27 years before, namely, the origin of Siebold's famous gynandromorphic bees from the Eugster hive. Boveri was able to secure the original material and to work it through in order to determine whether his old hypotheis of 1888 or those of Morgan (1905) or Wheeler (1910) was correct. By means of a very beautiful analysis he shows that his own hypothesis—fertilization of one nucleus after a premature division—is the only one in agreement with the facts.

It has already been said that Boveri's cytological work was always intermingled with studies in experimental embryology. His favorite objects, sea-urchin egg and *Ascaris* embryos urged him to work out problems in that line. There may be mentioned only two of his most successful pieces of work. One of these deals with the polarity of the sea-urchin egg. Selenka and Morgan were already acquainted with some of the facts, and the work of Roux, Driesch and Wilson had brought the discussion of egg-axes, regulation and equipotential systems, to the foreground. Boveri (1901) now is able to demonstrate morphologically the polarity of the sea-urchin egg—the well-known pigment ring—and to point out in a series of experiments how this preformed polarity explains all the previous results regarding the development of isolated blastomeres, fragmented eggs, deformed germs and larvæ with dislocated blastomeres.

The second series of experiments—partly done in connection with two of his students (Miss Stevens and Miss Hogue)—deals with the potency of the *Ascaris* blastomeres, studied especially with the centrifuging method and in cases of dispermia. His paper, "Die Potenzen der Ascarisblastomeren," in R. Hertwig's Festschrift, 1910, constitutes another high-water mark of his work. He mixes the plasmatic content of the eggs by centrifuging them and combines this in other cases with destroying one of the first blastomeres with ultraviolet rays. Then he follows with great accuracy the cell-lineage and reaches through a wonderful analysis the quite unexpected conclusion that in these eggs with strongly determinate cleavage nothing like organbildende Keimbezirke can be present, and that these eggs are very probably to be regarded as a "harmonious-equipotential system." In the same paper he gives an answer to another question, which had vexed him, since he first entered the field of cytology, namely, the cause of the diminution of the chromosomes in the somatic cells. By a most remarkable analysis he reaches the conclusion that the constitution of the protoplasmic surroundings is alone responsible for the process.

Besides all this closely correlated work, Boveri only once—with the exception of his doctor's thesis—entered a quite different field of research. The result was his paper on the nephridia of *Amphioxus,* one of the classics of vertebrate morphology (1892). His discovery of the protonephridia of that famous animal, as the result of logical thinking and consequent observation, is well known to every biologist as well as the phylogenetic significance attached to it. In his later years he returned but once to this subject, following Goodrich's discovery of the solenocytes, but always retained a special interest in all questions concerning the *Amphioxus,* encouraging also the work in this direction done by his assistant Zarnik.

The number of papers published by Theodor Boveri is comparatively small, only about forty. But of these there are very few which could be called unimportant, and a surprisingly large number of them constitute landmarks in the progress of our science. This is to be explained by his way of working and thinking. If his ability is to be characterized in a few words, one might say he was keen, philosophic and artistic. Keen, in that his piercing intellect immediately saw behind a minor observation its far-reaching consequences, and followed them patiently to the last detail. Philosophic, as he followed his discoveries and put them in their proper place within the science of biology with an exact logic, sometimes almost striving at dialectics, and with the spirit of clearness and order. And last, but not least, artistic. The construction of his ideas has an almost esthetical beauty. And at the same time he was a master of the language. If he talked before a learned society he succeeded, in spite of his calm, almost monotonous speech, to fascinate everybody, through the clearness and thoughtfulness of his words, as well as through the wonderfully refined diction. His papers are written in the same spirit; few scientific treatises have been better written. And where he could devote himself especially to the esthetic side of a paper, as in his wonderful Rector's address, "Die Organismen als historische Wesen" or in his necrologue on Anton Dohrn, he reached the state of literary perfection of a work of art. And these characteristics of his work were in full harmony with his personality. At first sight not remarkable, he immediately fascinated one through his eyes, flashing with genius. And those who knew him were aware how much the artistic side of life meant for him, who was more than an amateur in music and painting. He was not only a great scholar, but a noble, harmonious man. What he has been for our science may be said with the words that he himself dedicated to Anton Dohrn:

> Er brauchte ja nur um sich zu blicken, um sich sagen zu muéssen, dass er der Biologie einen Impuls gegeben hat, dem wenige sich an die Seite stellen können, und dass seine Tat und mit ihr sein Name leuchten werden in der Geschichte unserer Wissenschaft, weit hinaus, wo nur die hoechsten Gipfel noch sichtbar sind.

RICHARD GOLDSCHMIDT

OSBORNE ZOOLOGICAL LABORATORY,
YALE UNIVERSITY,
NEW HAVEN, CONN.

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THE REACTIONS OF THE MELANOPHORES OF AMBLYSTOMA LARVAE—THE SUPPOSED INFLUENCE OF THE PINEAL ORGAN

HENRY LAURENS
Osborn Zoological Laboratory, Yale University

SIX FIGURES

INTRODUCTION

Although the literature dealing with the chromatophores is very voluminous, nevertheless there are still many points concerning which our knowledge is far from complete. One of these, and a fundamental one, because it lies at the foundation of our comprehension of the physiology of the pigment cells, is the relation between them and the nervous system. We know much about the reactions to various stimuli of the chromatophores of many animals but when we come to compare them it is found that they are so diverse that it is almost impossible to lay down any general rule which will cover all cases. In some animals light causes a contraction of the pigment cells, in others it has no noticeable effect, and in still others it produces expansion. Attempts have been made, of course, to explain these different results, and in the excellent review by Fuchs ('14) this is often done, with, it must be admitted, not always marked success. The particular case in which we are interested concerns the reactions to light and to darkness of the melanophores of Amblystoma larvae.

Babak ('10) obtained very interesting results regarding the melanophores of these larvae. He found that there was a difference between the reactions of the pigment cells of normal and blinded Axolotl (A. mexicanum Cope, A. tigrinum Laurenti) larvae to light and darkness. In diffuse light, according to Babak, the melanophores of normal seeing larvae contract, those of blinded larvae expand. In darkness the melanophores of normal larvae expand, while those of blinded larvae contract. This opposite reaction of the melanophores of normal and blinded larvae he found, however, not to occur until the larvae had attained a certain stage of development, about 17.0 mm. long. Babak believes, (Laurens '15, p. 592) that before this period the retina has not acquired the pigment motor function which it later has, so that the melanophores simply respond to direct stimulation, which is therefore the same in both normal and blinded individuals. After this period, by means of the control which the eyes have gained through the central nervous system, the sense of the reaction of the melanophores is reversed, the effect of indirect stimulation through the eyes being opposite to that of direct. Babak's explanation of why there should be this difference between the reactions of normal and blinded larvae, or in other words, why the effect of indirect stimulation of the melanophores should be opposite to that of direct is briefly as follows (Laurens, '15, p. 623): the chromatophores of normal Axolotl larvae in both phases of their movement—expanding and contracting—are governed by the central nervous system, and this double innervation is conditioned upon the retinae which have opposite influences upon the nervous system according as to whether they are illuminated or darkened. The darkened retinae exert a positive influence on the chromatophores through the nervous system, just as the illuminated retinae do, but in the reverse direction. The destruction of the retinae has an entirely different result from that obtained by darkening them. In other words, the retinae in complete darkness are active and exert a positive influence which is directly opposite to that caused by illumination.

Babak, however, does not believe that these two opposite effects of the retinae upon the chromatophores are either of them inhibitory, but that they are two kinds of tonic influences. The impulse bringing about the expansion of the chromatophores originates in the darkened retinae, and is so strong that it overcomes the tendency of the darkened chromatophores to contract and brings about their expansion. On the other hand, the impulse for the contraction of the chromatophores originates in the illuminated retinae and is in turn so strong that it overcomes the tendency of the illuminated chromatophores to expand and brings about their contraction.

The results which were obtained from a study of the reactions of the melanophores of larvae of A. punctatum and of A. opacum (Laurens '15) were such that this explanation of Babak's could not be applied to them. They threw no doubt, however, on the assumption that both phases of the movement of the melanophores are normally under the control of the nervous system by means of the eyes, although one of the influences of the retinae must be admitted to be inhibitory, and opposite in effect to an impulse which causes the pigment cell to contract.

The results of my work showed that the melanophores of normal and eyeless larvae react primarily in identically the same way, expanding in light and contracting in darkness, the only difference being that the reactions come about more quickly in the normal than in the eyeless larvae (p. 585 and table 2). Secondarily, however, the melanophores of the normal larvae are found to be in the opposite conditions in both light and darkness to what they were in before, for after having been kept for from three to five days in light the melanophores are contracted, and after having been for five days or more in darkness, the melanophores are expanded. The melanophores of the eyeless larvae do not show these secondary reactions.

It was assumed (pp. 624–625), to explain these secondary reactions of the melanophores of normal seeing larvae, that, although the primary effects of indirect stimulation of the melanophores through the eyes were the same as those of direct stimulation, in the case of light the constant illumination or stimulation of the retinae had the result of causing impulses to be started, the end effects of which were opposite to those of direct stimulation and in this way the secondary contraction of the melanophores was brought about. These impulses were supposed to have their immediate cause in certain photo-chemical changes taking place in the retinae. In the case of darkness the same thing was supposed to take place, in that, due to the long continued absence of light, chemical changes in the retinae started impulses which reaching the melanophores caused them to secondarily expand. Briefly, the seat of the causes of the secondary changes were assumed to be in the retinae, for which there was abundant experimental proof, just as Babak assumed that the reactions of the melanophores of the normal larvae were due to the influence of the eyes, which were opposite in effect to direct stimulation of the melanophores themselves.

Now Fuchs ('14, p. 1545) considers that Babak's explanation of the differences between the reactions of the melanophores of normal and blinded Axolotl larvae is unsatisfactory and seeks to explain it by advancing a theory based on the results of von Frisch's work on the minnow Phoxinus (von Frisch '11, p. 374). As his chief objection to Babak's explanation, he points out that the latter's contention that the expansion of the pigment cell is as much an active process as its contraction, and that therefore the condition of rest is one of medium pigment contraction, is untenable. For all that is known concerning the distribution of pigment in the chromatophore makes it necessary to regard the expanded condition as that of rest, while a condition of medium contraction must be considered as the result of a tonic condition of excitation, no matter where the tonus arises.

Fuchs therefore offers the following explanation of Babak's results, seeking in the first place to show why the melanophores of young Axolotl larvae expand in the light. Substances, he says, which are perhaps products of inner secretions, but which at any rate are the results of the ordinary processes of life, arise and cause the melanophores to contract. Now if the young larvae are removed from all possibility of external stimulation, then under the influence of these metabolic products the melanophores contract. This is the reason why the melanophores are contracted in darkness. But if a light stimulus, at this time, be allowed to act on the parietal organ, then through the stimulation of this organ an impulse is started which inhibits the endogenously produced contraction and the melanophores expand. Gradually, as the larvae grow older, the eyes develop and gain an influence (i.e., a pigment motor function) over the pigment cells.

The stimulation of the retinae (illumination) produces a contraction of the melanophores, and as the eyes continue to develop and finally gain the upper hand in the sense life of the animal, so the impulses started by illumination of the retinae become stronger and finally overcome entirely the inhibitory influence of the parietal organ, so that in light the melanophores are contracted. Now, if the influence of the eyes is removed, by blinding the larvae, then the parietal organ is again in complete control, and the melanophores therefore expand in the light.

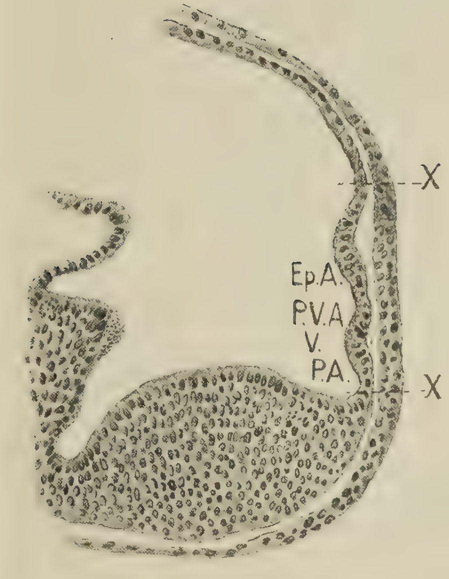
The remainder of this theory of Fuchs is as interesting as what has gone before. He goes on to say, that whether all larvae have a functional parietal organ, and whether the functioning power decreases as the development of the animal proceeds—which he considers probable—can be learned only experimentally. If this is found to be true then, he believes, it will be easy to understand why in adult animals the eyes have no particular influence over the color changes. For when the eyes have overcome the opposite influence of the parietal organ then after the extinction of its influence the function of the eyes must naturally in this respect also be less, because their antagonist, the stimulation of which causes the pigment cells to expand, is lacking. In other words, Fuchs would claim that the pigment motor function of the eyes, by means of which contraction of the pigment cells is brought about, is developed to offset an opposite effect of the parietal organ, and after this has been overcome it disappears. Moreover in his theory, nothing is said concerning the direct stimulation of the pigment cells by light, and no indication is given as to just how the chromatophores are stimulated after the influence of the eyes as well as that of the parietal organ has been extinguished.

EXPERIMENTAL

The work, the results of which will be given in the succeeding pages, was undertaken to put to experimental test this theory of Fuchs. More particularly to see whether the parietal organ in young normal seeing larvae can be shown to exert any inhibiting influence upon the melanophores, which later disappears after the pigment motor function of the eyes has developed, but which in eyeless larvae is still present, and remains so until the larvae reach a certain stage of development when, according to the letter of Fuchs' theory, it would be expected to decrease and finally disappear.

It should be mentioned, of course, that this theory of Fuchs was put forward to explain the results obtained by Babak concerning the reactions of the melanophores of the Axolotl larvae. As has been pointed out, these are different in certain points from those obtained by me with the larvae of A. punctatum and opacum. But the main fact with which Fuchs' explanation is concerned is the same, viz. that in larvae deprived of their eyes the melanophores expand when the larvae are illuminated. If his explanation, that this is so because of the stimulation of the parietal organ, by virtue of the fact that impulses going out from it inhibit an endogenously produced contraction of the melanophores, holds for larvae of the Axolotl, it must hold also for larvae of A. punctatum and opacum. The same applies to his belief that when the eyes are present their stimulation starts impulses which are opposite in effect to those sent out by the parietal organ and stronger, so that these are rendered of no avail.

To test this hypothesis of Fuchs several methods of experimentation were employed and these will be taken up in order. But before proceeding to describe and discuss their results it seems desirable that some idea be had concerning the development of the so-called parietal organ, and its position at this time and later. To do this it is hardly necessary to more than call attention to the accompanying figures. In figure 1, which is a view of the extreme anterior portion of a sagittal section of a 5.3 mm. larva of A. punctatum there is seen the beginnings of the development of the epiphysis and paraphysis. In figure 2, of a 6.6 mm. larva, development has proceeded only a little further. In figure 3, which is of an 8.0 mm. larva, the epiphysis and paraphysis are both seen as evaginations, the former of the diencephalic wall, the latter of the telencephalic, the two cavities of the brain being partially divided by the velum transversum. In figures 4 and 5 (of larvae 14.5 and 34.0 mm. long respectively), the later development of these parts of the roof of the brain are shown. The condition seen in figure 5 is typical of older larvae and of adult Amblystoma, although it must be said that the opening into the stalk of the epiphysis cannot always be made out with great distinctness, so that there is a question as to whether it may not later be solid instead of hollow as here represented. In no case, however, was the epiphysis found not attached to the brain. Its cavity is more or less incompletely divided by septa.



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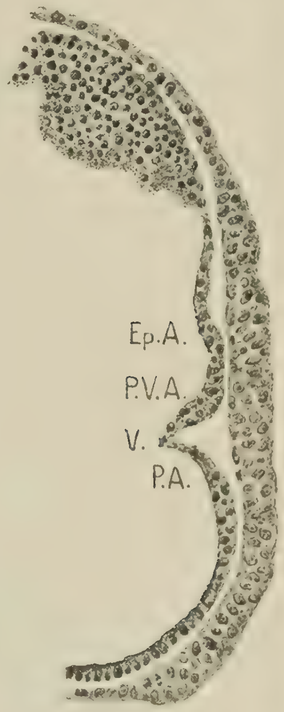

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Fig. 1 The anterior end of a sagittal section of a 5.3 mm. larva of A. punctatum. *Ep.A.*, epiphysal arch; *P.A.*, paraphysal arch; *P.V.A.*, post-velar arch; *V.*, velum.

Fig. 2 The anterior end of a sagittal section of a 6.6 mm. larva.

The paraphysis, which is much larger and more conspicuous than the epiphysis, has a wall of a single layer of cells and a large irregular cavity with branching tubules with which the blood vessels of the choroid plexus are in intimate relation.

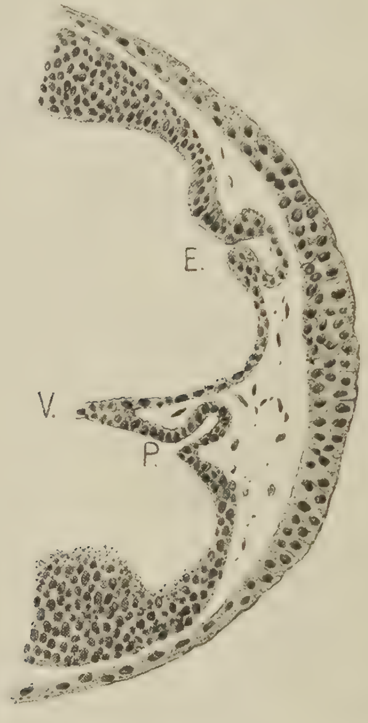

Fig. 3 The anterior end of a sagittal section of an 8.0 mm. larva. *E.*, epiphysis; *P.*, paraphysis; *V.*, velum.

These figures also serve the purpose of showing that the epiphysis, as in other Urodeles, is relatively poorly developed in Amblystoma and moreover that there is no pineal or parietal organ or eye. The paraphysis on the other hand reaches a high degree of development and with the surrounding blood vessels can be seen through the skin and brain case of the larvae, particularly when the melanophores are contracted or when they are scarce. Figure 6 is given to show this, where, it will be admitted, it has very much the appearance of a brow-spot or "Scheitelfleck" and might be taken for such.

From reading over the literature one is soon forced to the conclusion that it seems doubtful whether a parietal organ in any Urodele could have an influence on the reactions of the melanophores, and this particularly for the reason that a parietal organ as such does not exist. There seems to be in all a small epiphysis (Studnička '05, and Warren '05). Nevertheless, although there is no parietal organ and only a small epiphysis, there was still the chance, considering the results obtained by von Frisch ('11), that in this region of the brain there might be a 'center' the stimulation of which would have an inhibitory influence on the melanophores.

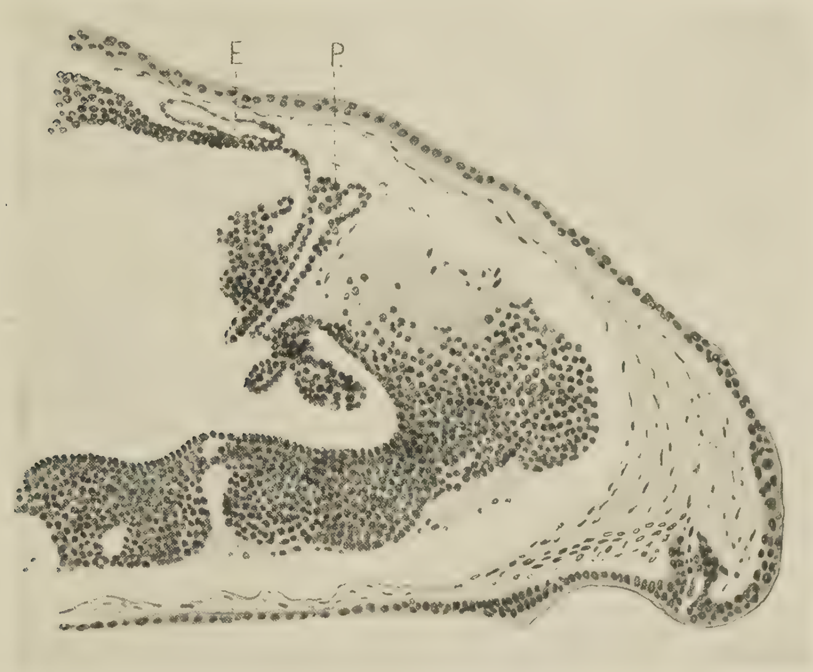

Fig. 4 The anterior end of a sagittal section of the head of a 14.5 mm. larva.

The first of the methods of experimentation and the one which seemed at first thought to be the most promising, but which failed for reasons which will be given later, was to remove that portion of the undifferentiated nervous system from which the epiphysis arises. This was accordingly carried out on larvae measuring between 5.5 and 6.1 mm. The operation is a simple one requiring but slight practice. The embryo to be operated on was placed in 0.02 per cent NaCl in a watch glass, the bottom of which was covered with paraffin. In this a little pocket was dug near the middle of the dish and into this the embryo was slipped so that the anterior end was directly upwards. Under the binocular microscope, with a small pair of iridectomy scissors, the epidermis and then the desired portion of the undifferentiated nervous system was removed. The extent of the portion removed is indicated approximately in figure 1 between the dotted lines x—x. This operation was performed on 10 embryos between 5.5 and 6.1 mm. long. On 10 others in addi-

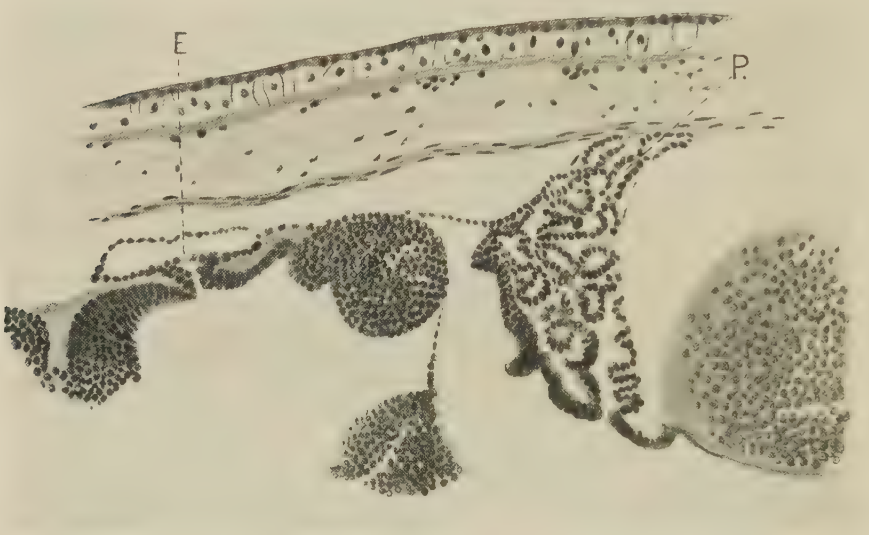

Fig. 5 A sagittal section of a portion of the head of a 34.0 mm. larva to show the position and relative development of the epiphysis and paraphysis.

tion the optic vesicles were also removed by the method described in former papers (Laurens '14, p. 196 and '15, p. 579).

A complete series of experiments were carried out on these 20 larvae together with control normal seeing and eyeless larvae, for the purpose of seeing whether there was any difference in their reactions to darkness, light and backgrounds and in the time when these reactions first made their appearance (Laurens '15, pp. 583–595). The results were in complete agreement with my earlier ones. But unfortunately upon sectioning the

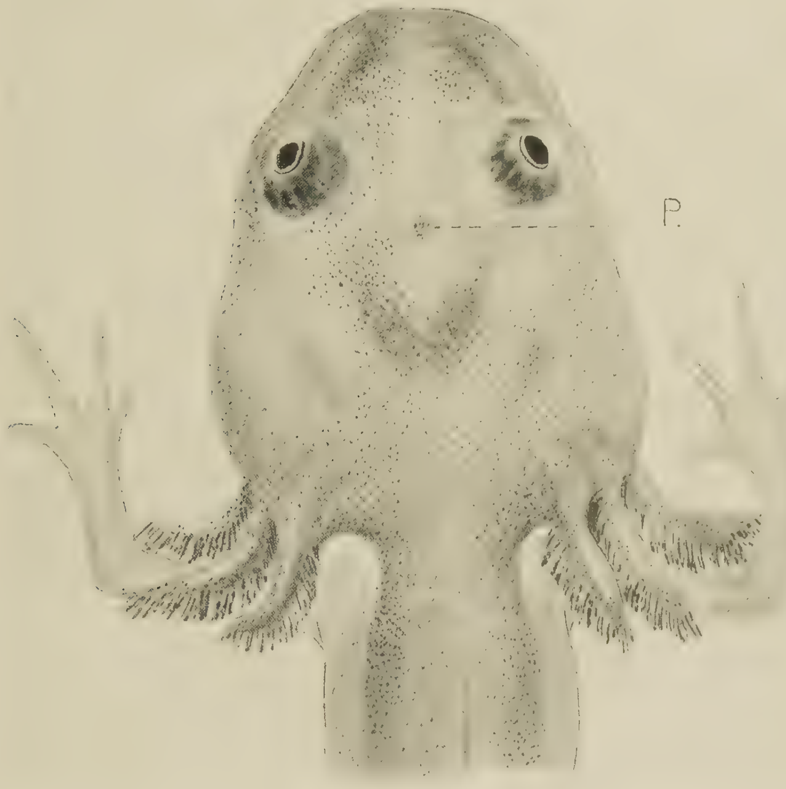

Fig. 6 The anterior end of a 42.0 mm. larva to show the appearance of the paraphysis (with the surrounding blood vessels) as seen from the outside.

larvae and examining the brains it was found that in nearly every case either complete or partial regeneration of the parts concerned had taken place. In some cases only a small epiphysis was present, while in others no difference between this region in the operated larvae and in the normal larvae could be detected. In only 2 larvae sectioned was there no sign of an epiphysis. Therefore another method of getting rid of the epiphysis, etc. had to be resorted to.

This was to remove it from older larvae after differentiation of the nervous system had taken place. This operation was successfully carried out on 28 normal larvae ranging in length from 12.5 to 40 mm., and in 8 eyeless larvae ranging in length from 12.0 to 30.0 mm., from which the optic vesicles had been removed when they were about 5.5 mm. long. In addition to removing the epiphysis the roof of the diencephalon was also cut out in 3 normal and 3 eyeless larvae when they were about 12.5 mm. long.

After the experiments to test the various reactions of these larvae to darkness, light and background had been finished several of them were killed, sectioned and the brain examined for indications of regeneration of the parts involved. Some of the others were allowed to live until they had metamorphosed, others not quite as long. Eventually they were all of them sectioned and studied. In only one was there any sign of an epiphysis.

The carrying out of the operation necessary to remove the epiphysis is not a particularly difficult one. The larvae were anaesthetised by placing them in 0.02 per cent chloretone. With a pair of iridectomy scissors a flap of skin was cut and left attached at one end so that it could be folded back, after which the roof of the brain case was removed in small pieces until the desired portions of the brain were sufficiently exposed to be removed. Out of a total of 43 operations 28 normal and 8 eyeless larvae survived and grew.

Again the experiments carried out on larvae operated on in this way showed no differences between those which have already been reported in such detail for normal and eyeless larvae. In addition to simply placing the larvae in darkness and in light on various backgrounds there was carried out a series of experiments in which a narrow beam of light, reflected from a Nernst glower by a mirror and concentrated with a lens, was thrown on the region of the brain from which the epiphysis arises. The results of these experiments give additional evidence concerning the ability of the melanophores to respond to direct stimulation by light.

When Amblystoma larvae are placed in darkness and observed by means of a faint red light, they remain motionless for the greater part of the time if there are no other larvae present or other animals which may serve as possible food. It is therefore a simple matter to throw such a beam of light upon any portion of the dorsal or lateral surface of the body and keep it there. Naturally, at times the larvae move by alternately crawling and swimming with the snout close to the bottom of the vessel in the characteristic nosing fashion, nevertheless, the animals can usually be followed with the narrow beam of light until they again come to rest.

It was found that in no case when the beam of light was thrown upon the region of the epiphysis of normal seeing or eyeless larvae did an expansion of the melanophores over the whole body take place. On the other hand the result of such local illumination at any place on the body results in an expansion of the melanophores stimulated. These experiments are rather tedious because of the time consumed in carrying them out. In table 2 (Laurens, '15, p. 585) it will be seen that it takes from 1½ to 2 hours for the melanophores of normal larvae with eyes to expand when the larvae are placed in the light, and 2 to 3 hours for the same thing to happen in eyeless larvae. In these experiments with local illumination no expansion was observed taking place in less than 2 hours and in most cases an illumination lasting 3 hours or more was necessary.

These experiments on local illumination were carried out on both normal and eyeless larvae with and without the epiphysis and the roof of the diencephalon. In some of them not only was the beam of light thrown on the region of the head under which the epiphysis is, but the brain was exposed and directly illuminated. In addition it was also carried out on larvae in which the central nervous system had been completely destroyed by boring it out. In all, the results were the same, expansion of the melanophores illuminated by the beam of light, and no effect on the pigment cells of the remainder of the body.

These results show conclusively that the expansion of the melanophores of the larvae of A. punctatum caused by light are due primarily to the direct stimulation of the pigment cells themselves and not to the inhibitory action of the nervous system. The nervous system certainly helps to bring about the expansion, just as it helps to bring about the contraction of the melanophores when the larvae are placed in darkness, for, when the reaction times of normal and eyeless larvae are compared it is seen that those of the former are shorter (Laurens, p. 585, table 2). The experiments on larvae in which the central nervous system was cut through, and partially or totally destroyed (pp. 614–616) could be considered sufficient proof against the assumption of the action of an inhibiting center in Amblystoma, such as Fuchs suggests. But there seemed sufficient reason for carrying out experiments to test just this point. These have afforded us additional evidence concerning several things mentioned in my former paper and have added information not there contained. One interesting fact which was brought out and which seems worthy of remark is that although the roof of the diencephalon is cut out with the epiphysis, nevertheless the secondary reactions of the melanophores, and the reactions to background, which are certainly dependent on the presence of the eyes, still come about. As we know, the median portion of the diencephalon has no nervous elements in it, these being in the lateral walls. In a few experiments which were carried out on larvae after cutting the lateral walls, the secondary reactions of the pigment cells, as well as the reactions to black and white backgrounds did not take place.

DISCUSSION AND CONCLUSIONS

Fuchs' idea that the parietal organ, or, as we may now say, the epiphysis, and the surrounding region of the roof of the brain has an inhibitory influence on the melanophores does not hold for the larvae of Amblystoma punctatum. It has already been pointed out that Babak's explanation of the reactions of the melanophores of the Axolotl larvae also cannot be applied to those of the melanophores of A. punctatum.

When one considers the relative anatomical insignificance of the epiphysis in the Urodeles (Studnička, '05 and Warren, '05) it is hardly surprising that this organ should be found to have no influence upon the melanophores. In the teleosts and particularly in the reptiles, the epiphysis reaches a high degree of development with a distinct parietal organ, so that in these animals it is possible that it may have an important function in connection with the reactions of the melanophores (Fuchs, pp. 1442 and 1651, and von Frisch, '11, p. 374). Nevertheless, it cannot be assumed from the results of von Frisch's experiments, —which are the only ones that have been carried out previously to specifically test this point—that even in the minnow Phoxinus, the parietal organ is responsible for the inhibiting effect produced by stimulation with light. For he found even after the parietal organ had been extirpated (controlled by microscopic sections) that stimulation with light of this region still produced an expansion of the melanophores, while shading it caused the melanophores to contract. Von Frisch further found that when the portion of the roof of the brain which extends from the point of junction of the epiphysis with the brain to the posterior commissure is also removed, that illumination may still sometimes result in an expansion of the melanophores, although sometimes there is no change at all. From these results von Frisch is finally forced to conclude that in the region of the diencephalon there must be light perceiving cells which function as an inhibiting center, and from which nerve fibers run to the deeper portions of the brain which are in this way connected with the pigment motor apparatus so as to bring about this reaction of the melanophores. Perhaps, he thinks, these cells are particularly numerous in the parietal organ, perhaps they are identical with the sense cells and perhaps the connecting nerve fibers constitute the 'Tractus pinealis.' But they are not limited to the parietal organ, else, when it was removed, the reactions of the melanophores would cease to take place.

As an interesting fact von Frisch found this region of the roof of the diencephalon to be also particularly easily stimulated by other means than light, e.g., electric currents (pp. 335, 336, and 376) in this particular fish Phoxinus.

Von Frisch (p. 377) is led to make the suggestion, later taken up by Fuchs, that perhaps in other animals the parietal organ may be found to have an influence on the pigment cells and that thereby an explanation may be found for some of the perplexing diverse results, e.g., in tadpoles, where darkness causes the melanophores to contract and light causes them to expand, while the opposite reactions take place in the adult frog, and in Axolotl larvae, citing Babak's results on normal and blinded individuals. Also for the reactions of the melanophores of Salamandra maculosa larvae and of Triton cristatus adults which, however, show an expanded condition of the melanophores in light and in both normal and blinded individuals.

However, he has himself (p. 378) carried out experiments with Salamandra larvae and adult Tritons with different results than he hoped for. For in these animals illumination of the head region did not give as clear results as in Phoxinus. Furthermore he received the impression that local illumination of any portion of the body results in an expansion of the melanophores of the whole body. In other words the illumination of the portion of the body under which the epiphysis is has no different effect from illumination of any portion of the body.

Experiments such as von Frisch suggests should be carried out on tadpoles and frogs, in some of which the parietal organ has been destroyed, in others of which the influence of the eyes has been removed, etc. In these animals the pineal organ attains a comparatively high degree of development (Studnička, p. 110 ff.) Furthermore these results should be compared with those obtained with such an animal as Diemyctylus, where the reactions of the larval melanophores are also opposite to those of the adult, but where there is no pineal organ and where the epiphysis is not highly developed. The results of such experiments would certainly afford a great deal of evidence concerning the influence of the parietal organ on the melanophores. Such experiments should also be carried out on reptiles, where the parietal organ reaches a high degree of development, and in some animal where the color changes of the skin are well marked.

The nervous system must be admitted to exert a very important effect upon the chromatophores of any animal, and this effect is for the most part conditioned upon the eyes, which have been shown to play such an important part in the color changes of many animals (see, for example, the reactions which have been described for the melanophores of Amblystoma, or the effects observed by von Frisch, '11 and '12, of blinding fish). Take away the eyes and the major part of the controlling influence of the nervous system over the pigment cells of the skin is lost. This of course does not apply in full to those cases where the eyes have, or are believed to have, lost control over the chromatophores and where other things than light, such as temperature, tactile stimuli, etc., are believed to be of greater importance.

But even in such a case, as Biedermann has shown, the reactions of the melanophores to light are controlled by the nervous system in that this exerts a tonic influence upon the pigment cell. Biedermann demonstrated that the pigment cells of the frog as soon as they are released from the influence of the nervous system have the tendency to expand. When he cut through the connection between the thalamus and the midbrain in Rana esculenta and R. fusca, as well as in Hyla, he found that the melanophores expanded, and that when the frogs (particularly Hyla) operated on in this way are kept for weeks in darkness as well as in diffuse light no change takes place in the melanophores. Briefly, that light has under these conditions little, if any, effect on the melanophores, unless direct sunlight is used.

It is my belief that the general function of the chromatophores is to expand when illuminated and to contract again when in darkness. But when the melanophores are under the influence of the nervous system, which they normally are, it may be, owing to the nature of things, environmental conditions (background) or psychic factors, etc., that the sense of the reaction to a light stimulus is reversed and contraction is brought about.

What causes the melanophores to contract in darkness is another matter, it happens whatever its cause may be. From the conflicting evidence that we have we are forced to speculate regarding its cause. It may be due to chemical substances, such as Fuchs suggests, which arise as a result of the ordinary processes of life (inner secretions, or what not). It may be due to chemical changes in the skin, brought about by darkness, (or the absence of light). It may be due, on the other hand, in some cases at least, to what may be called for want of a better term, a simple relaxation, for the reason that when pieces of the skin are removed from some animals (the frog, Biedermann; Anolis, Carlton) the melanophores contract. (Laurens, '15, p. 599). But that this contraction may also be due to chemical changes is perfectly possible.

The expanded condition of the melanophores in darkness which comes about in animals in which, due to the control of the nervous system, the pigment cells contract in light, is probably not a specific reaction to darkness but one which is due to the lack of the nervous impulse which light sets up. The pigment cells have become subordinated to the influence of the nervous system, and when the condition of excitation ceases (in darkness) the melanophores expand. It is interesting, in this connection, that Hargitt could observe, in tree frogs, no definite effect of darkness (and of low temperature) on the pigment cells.

The contracted condition of the pigment cell is claimed by many to be its active state. Arguments are put forward to support this view from the fact that muscle contracting stimuli also cause the chromatophores to contract. But light (and under certain conditions high temperature) is the adequate stimulus for the chromatophores of animals and light causes the melanophores in many animals to expand, both when under the control of the nervous system and when released from this control, perhaps in all when the influence of the nervous system is removed. The fact that Hertel ('07) obtained contraction of the chromatophores when he locally stimulated the skin of Triton larvae with ultra violet rays is another matter, since these rays probably always cause the pigment cells to contract (Laurens, '15, p. 599). That he also obtained contraction when he locally stimulated with yellow and red light, may be due to one or more of several things. First, that the intensity of the light was high; second, since the light was focussed, that the pigment cells were subjected to a high temperature; and third, that the contraction of the pigment cells stimulated was a reflex action. This last possibility has less in its favor than the others, since there was no indication of spreading. Ballowitz's ('14) results of contraction are probably due to the effects of the high intensity of the light that he used, about 1000 candles, hardly to a heat effect (see p. 200). Moreover the melanophores that he experimented with were taken not from the skin but from the "Hirnhaut." Finally the results of Hooker ('12) are also, it seems to me, capable of explanation.[1] Hooker found, when the melanophores of the frog (R. fusca) were completely deprived of their nervous connections, either in the body or when bits of skin were placed in hanging drop cultures, that for a day the reactions of the melanophores were the same as when they were under normal conditions. But after this period of time had elapsed the reactions of the pigment cells to light and darkness were reversed. Hooker offers no explanation of this curious fact. An explanation for the primary reactions, those lasting for the first day, may be found in the fact that sunlight was used as the source of light. It is therefore highly probable that the contraction of the melanophores was a heat effect and not due to the light at all. When the melanophores are placed in darkness the pigment expands again, not because of a specific stimulating effect of darkness, but simply because the heat stimulus ceases to act. Why, after a day, these isolated melanophores show exactly the reverse reactions to a heat stimulus that they did before is not entirely clear to me. That it may be due to an increase in the acidity of the cells, both of the pigment and surrounding tissue cells, in other words to a change in the H ion content, is not absolutely impossible.

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[1] Opportunity is taken to call attention to the fact that Hooker is misquoted on p. 598 of my former paper ('15) though rightly so on p. 628. Also that Ballowitz is incorrectly quoted on p. 598. The statement concerning his results should read that when the melanophores are removed from the body they contract, but when placed in salt solution they partially expand. When they are now illuminated they contract. The opportunity is also taken to point out a mistake in table 4, p. 589 where under 'black background' a II. reaction is indicated as follows: "contraction (3–4 hrs.)" This should be struck out.

Steinach's results, judging from the results of Biedermann's work which show the dependence of the chromatophores upon the central nervous system, were probably also obtained by illuminating the animals with sunlight. The effects therefore are to be referred to the action of heat and not to light.

The chief function of the chromatophores is probably to enable the animals possessing them to adapt themselves to the intensity and color of their background. To make this possible the pigment cells must be under the control of the eyes which regulate through the nervous system, the movements of the pigment suspended in their cytoplasm. It is very possible that in some animals the eyes may have no (or very little) influence over the chromatophores as Biedermann has pointed out to be the case in the frog where according to him, light plays a small part in the color changes. In some animals conditions are of course different from those in others. Von Frisch ('11 and '12) has shown this to be the case in fishes. The minnow Phoxinus does not possess in any marked degree the power of adaptation to background, but the melanophores respond more to the relative intensity of the light. In Salmo the response of the melanophores to different backgrounds is very marked. In Crenilabrus pavo this is also the case. But the control of the nervous system is in each of a different nature. In Phoxinus when the eyes are removed the melanophores expand in the light and contract in the dark, which is opposite to the conditions seen in the normal fish. In Salmo, when blinded, the melanophores are under all conditions expanded, and in Crenilabrus the melanophores expand in the light and contract in the dark just as they do in the normal fish, only more extensively.

The reactions of Amblystoma larvae to different backgrounds show that this is an important function of the melanophores. Amblystoma larvae are nearly always found in pools in the woods in which the bottom is covered with leaves which are either dark brown or black. Occasionally they may be found in ditches in a field, but here the bottom is of black mud. Over such a background, as my experiments have shown, the larvae are very dark, almost black, due to the complete expansion of the melanophores. But over a white background the larvae are pale, exhibiting, to be sure, not such a marked adaptation, but at any rate the melanophores are contracted. Also over an indifferent background, when the illumination is constant and bright, the melanophores contract ($\frac{1}{8}$ to $\frac{1}{4}$ expansion), so that the larvae are more nearly of the color of the bottom. The fact that the time required for the changes in the melanophores to come about is long and that therefore the usefulness to the individual in the way of protection, of the ability of the melanophores to change according to the background, is of doubtful value, is an argument that can be answered by the statement that probably such conditions in change of background are not very likely to occur in nature. But the fact that if they do occur the melanophores can react to the change shows that the ability to change is in the nature of an adaptive one. I have carried out experiments in a large aquarium, directly in front of a window in my room, to further test this matter. In one series of experiments the bottom of one half of the aquarium was of black mud or of black leaves, the other half of whitish sand, the larvae being free to swim from one to the other. Under these conditions the larvae are of course not at all adapted to the background for they do not remain in any one portion of the aquarium long enough for the melanophores to react adaptively. But if the aquarium is divided by a glass partition placed at the dividing line between the two different colored bottoms then the adaptive changes of the melanophores which have been described, do come about and the coloration of the larvae is strikingly similar to the bottom, particularly in the case of the black condition, so that they are very difficult to see. If the partition is now again removed so that the larvae can swim through the whole aquarium freely then the difference in coloration between the larvae that have been over the one or the other background is very apparent.

The theory that the melanophores in an aquatic animal can have any function in regulating the temperature has been shown by Bauer ('14) to be highly improbable. Also it does not seem possible to suppose that the contraction or expansion of the pigment cells in the skin has anything to do with the reception of a light stimulus by sensory nerve endings. It is well known that phototactic reactions can be induced in animals, which have been deprived of their eyes, by illuminating the skin. The amphibians show this faculty very well (Parker '03, Laurens '11). But as Parker ('09) has shown only a very few fish show this ability on the part of the integumentary nerves stimulated by light to result in phototactic responses. These reactions are supposed to be started by the action of light on receptors in the skin, either directly as light energy (heat) or indirectly as chemical energy set free by the photochemical changes started by the action of the light on the skin, which stimulate the sensory nerve endings. The fact that in most fishes no such reactions take place, although the pigment motor function is well developed, argues against the pigment cells having any such universal function in aiding or retarding the rapidity of perception. Experiments were carried out by me ('14) to test just this point as to whether the condition of the pigment cell—contracted or expanded—had anything to do with the sensitiveness of eyeless Amblystoma larvae to light with negative results, and the conclusion was reached that the condition of the pigment in the skin melanophores had nothing to do with the sensitiveness of the larvae to light, although it was dependent upon the fact as to whether they had been kept in light or in darkness before their phototactic reactions were tested.

To conclude then this brief and fragmentary discussion it seems to me that the reactions of the pigment cells in the skin to light are in most cases adaptive. The primary reaction of the melanophore is to expand when illuminated which it always does when directly stimulated with light of ordinary intensity and sometimes when the stimulus is indirect and through the eyes. Fuchs (p. 1651) closes his review of the color changes of the reptiles with the statement that in those animals which do not show expansion of the pigment in the light, either the parietal organ has lost its function in the course of phylogeny or ontogeny, or the eyes have gained a regulatory influence over the light reactions which results in changing the original light reaction (expansion) into the directly opposite reaction (contraction). I should change this statement and applying it to the chromatophores of all animals say, that in those animals in which the pigment cells do not expand in light of ordinary intensity and under normal conditions of temperature but contract, this is due to the controlling regulatory influence of the eyes, by means of which the reactions of the melanophores are made adaptive. In other words, when the pigment cells contract in the light, this reaction is one that is bound up with the nervous system in that an impulse started in the retinae is sent out which is opposite to that produced by direct stimulation. Such is the case in frogs, in Diemyctylus, and in Phoxinus, Salmo, etc. Usually, however, the melanophores expand in the light, due in most part, to their direct stimulation, although the eyes still show an influence in that in many cases the expansion is not maximal as it is when the eyes are removed, and that when the eyes are present the reactions come about more quickly.

SUMMARY

1. The epiphysis of Amblystoma punctatum larvae has no influence on the reactions of the melanophores to light and darkness. There is no parietal organ.

2. The view is expressed that the reaction to light of ordinary intensity of the pigment cells in the skin of animals is to expand. When this does not take place it is due to the controlling regulatory influence of the eyes.

3. The reactions of chromatophores are believed to be adaptive in that they either respond to the relative intensity of the light or to the color and intensity of the background.

4. The control over the melanophores which the eyes possess is of course most important, for by this means the reactions of the melanophores are able to be adaptive.

5. Indirect stimulation of the melanophores through the eyes is not by any means always opposite in effect to direct stimulation, but only so when the conditions are such that it is necessary that the chromatophores contract in the light in order that the reaction be adaptive.

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EVIDENCE PROVING THE MELANOPHORE TO BE A DISGUISED TYPE OF SMOOTH MUSCLE CELL

REYNOLD A. SPAETH

Osborn Zoölogical Laboratory, Yale University

TWO FIGURES

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PRELIMINARY

The melanophores of the lower vertebrates, fish, amphibians and reptiles, are described by most authors as modified connective tissue cells (Fuchs '14). The evidence for such a conception rests chiefly upon a morphological basis. Leydig ('89, '92), showed that no sharp morphological distinction exists between unpigmented, connective tissue cells, inactive, pigmented connective tissue cells and chromatophores (melanophores) containing actively migrating pigment granules.

During the past four years I have been carrying on a series of experiments on the direct physiological responses of the melanophores in fish, chiefly in Fundulus. These experiments have presented an opportunity for occasional morphological and embryological observations also. In attempting to categorize any type of cell it is necessary to consider not only its morphology, but its development and physiology as well. An examination of many data of previous investigators, supplemented by my own observations has led me to the conclusion that the melanophores of lower vertebrates do not belong in the category of connective tissue, but are to be considered functionally modified smooth muscle cells. The object of this paper is to summarize the evidence for my position.

MORPHOLOGICAL EVIDENCE

For our present discussion the following occurrence of involuntary or smooth muscle in vertebrates is of importance, viz., the digestive tract, the vascular system, the eye and the integument. The units of which smooth muscle tissue is composed consist of elongated or spindle-shaped cells which may be branched or forked, containing a single or several oval or elongate nuclei. In certain cases (sphincter pupillae) the cytoplasm is filled with a dense mass of melanin pigment granules. We may therefore say with Franz ('06), (p. 452): "Es ist daher wohl eine zulässige Ausdrucksweise vom Sphincter als von einer Anhaüfung spindleförmiger Pigmentzellen zu sprechen."

In the lower vertebrates the melanophores occur chiefly in the derma, more rarely in the epidermis (Pouchet '76, in Rhombus; Müller '60, Eberth '67, Pouchet loc. cit., Biedermann '92 and many others in the frog; Schmidt '12, in Phelsuma), on the mesenteries and peritoneum and associated with the blood-vessels. Morphologically we find an entire series extending from relatively simple cells with two or three processes to the familiar, extensively branched forms found in the derma. Zimmerman ('93), for example, noted elongated melanophores between the fin rays in several adult teleosts and I have observed similar simple cells in the tail region of adult Fundulus and other teleost species. Franz ('08) has recorded the same condition in the edges of the fins in the larva of Pleuronectes platessa. On the surface of the iris in the eel Steinach ('92) has observed a transition from the stellate melanophores to the spindle shaped cells of the sphincter pupillae. He says (p. 515): "Die verästigten sternförmige Pigmentzellen . . . gehen dicht am Pupillarrand in einen Ring von besonders dunkeln Pigmentzellen über deren plumpe Körper sich mehr der spindelform nähern."

We may conclude then, that, as regards external and nuclear form and a melanin pigment content, no sharp morphological distinction can be made between melanophores and the smooth muscle cells of the sphincter pupillae.

EMBRYOLOGICAL EVIDENCE

Smooth muscle may be formed either from the mesoderm (mesenchyme), the commonest source, or from the ectoderm, (Nussbaum, M. '01).

Investigations upon the development of the dermal melanophores show a satisfactory agreement. With a single exception[1] they have been found to originate from mesodermal cells (Wenckebach '86, Bolk '10: Ehrmann '96, Eycleshymer '06, Weidenreich '12: Kerbert '77, Todaro '78, Zenneck '94). The chief controversial point in the case of the epidermal melanophores has been as to whether these cells develop from the epidermis in situ, or whether, as unpigmented mesenchyme cells, they wander into the region of the epidermis and become pigmented secondarily. In the first case their origin would be ectodermal and in the second, mesodermal. My own observations are limited to several species of teleosts. In the case of Fundulus, epidermal melanophores do not occur, but the origin of the dermal melanophores from wandering mesenchyme cells has been clearly demonstrated by Stockard ('15). Dr. Stockard has very kindly shown me a number of his own preparations and unpublished drawings of the developing melanophores in Fundulus embryos. His studies, made in connection with the developing circulatory system, have shown that in this species the yolk melanophores first appear as wandering, actively amoeboid, unpigmented mesenchyme cells. From the region of Kupffer's vesicle there develops a fan-shaped area of migrating cells which rapidly spread out on the surface of the yolk. These cells eventually give rise to connective tissue, the yolk melanophores, the endothelial cells and possibly also to the smooth muscle cells in the walls of the larger blood vessels. For some time after the pigment has begun to develop within the wandering cells that are destined to become melanophores, they continue to show active amoeboid movements. In a transparent pelagic egg like that of the scup (Stenotomus crysops), these wandering cells at the posterior end of the embryo may be observed with startling clearness.

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[1] Borcea, '09.

It is impossible to draw any sharp morphological or embryological distinction between certain pigmented connective tissue cells and chromatophores (Leydig loc. cit.) just as there are morphological transitions between connective tissue and smooth muscle cells (Flemming '97). We can say, then, that typical connective tissue cells, melanophores and smooth muscle cells are all known to develop from mesenchyme and furthermore, that smooth muscle cells (sphincter pupillae) and possibly also melanophores (Borcea loc. cit.) may develop from the ectoderm. The embryological evidence is thus at least not negative.[2]

PHYSIOLOGICAL EVIDENCE

By far the most striking evidence of the parallelism between smooth muscle and melanophores is furnished by physiological experiments. Franz ('06) first recorded the striking similarity between the physiological responses of the sphincter pupillae in Acanthias and the dermal chromatophores of the frog. He noted 1) that both respond to light by contraction, 2) that indifferent gases like N and H elicit no contractions of the chromatophores and that these gases prevent the contraction of the sphincter pupillae upon illumination; 3) that after death, the pigment cells and sphincter are both contracted. He then says (p. 453): "Wir können also in mancher Hinsicht Analogien zwischen den

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[2] It is certainly not without interest in this connection that the chromatophores of cephalopods have been found to develop from single smooth muscle cells by a complicated metamorphosis (Chun '02).

Sphincterfasern und mesodermalen Chromatophoren aufstellen;" and again: "Denn es scheint in mehr als einer Beziehung, dass die Chromatophoren nichts anderes als verkappte Muskelzellen sind."

Many of the older investigations upon the responses of the melanophores to various stimuli were carried out upon living animals. Here the situation was exceedingly complex since so many physiological factors had to be controlled. In a previous paper ('13 b) I have called attention to this objection and have described a method whereby the melanophores of teleosts, especially Fundulus heteroclitus, may be removed from the fish and stimulated in a variety of ways without the slightest mechanical injury in manipulation. The technique is exceedingly simple, consisting of the careful removal of the scales with their superficial sheets of dermal melanophores. These scales are readily transferred from one solution to another and, by selecting adjacent scales from the same fish, we obtain very satisfactory physiological units whereby it is possible to test the effects of a series of solutions—the degree of expansion or contraction of the pigment serving as an indicator of relative stimulation. In preparations of this sort in which the circulation and nervous control have obviously been eliminated, I have found that chemical stimuli such as 0.1 N KCl, heat (30° C.), ultra-violet light and induction currents all bring about a more or less rapid contraction of the pigment granules. Furthermore, in indifferent media like Ringer's solution or olive oil, the melanophores remain expanded for long periods. It therefore seems justifiable to consider the contracted phase that of stimulation, since the melanophores respond by contracting to the above familiar series of physiological stimuli. Throughout the following discusssion I shall consider the contracted phase of the melanophores as corresponding, physiologically, to the contraction in smooth muscle. The objections to the application of the term 'contraction' in the case of the melanophores, I shall take up at the close of this section.

I. Innervation

The activity of vertebrate smooth muscle is normally controlled through fibers of the sympathetic nervous system. Voluntary motor connections do not ordinarily occur.

The innervation of the melanophores has been satisfactorily demonstrated both histologically (Ballowitz '93) and physiologically (Pouchet '72 and '76, Von Frisch '11, Spaeth loc. cit.) in several species of teleosts. v. Frisch has recently corroborated and amplified the original observations of Pouchet, who first claimed the innervation of the melanophores to be sympathetic. I have repeated the striking experiments of v. Frisch upon Phoxinus with Fundulus. In this experiment one of the two branches of the sympathetic system is severed immediately behind the body cavity in the region of the haemal arch. Fish so treated lose their power of color adaptation posterior to the point of incision on the operated side, but continue to show normal motor responses. Reciprocally, severing of the spinal cord eliminates motor responses but, provided the operation has been carefully performed, the sympathetic adaptations to different colored bottoms remains normal.

In Fundulus I have also found at the base of the medulla a 'contraction center' corresponding with that found by Lode ('90) in the trout and by v. Frisch ('11) in Phoxinus. A lightening of the entire body of the fish follows the electrical stimulation of this center.

No satisfactory histological demonstrations of the nerve endings in the melanophores of amphibians and reptiles have been recorded. Bimmerman ('78), Biedermann, loc. cit. and more recently Hooker ('12) have however, all demonstrated, physiologically, the sympathetic innervation in the melanophores of the frog and Carlton ('04) has made similar observations in Anolis. There is thus a very satisfactory unanimity of opinion on this question.

II. Effect of Light

Through the investigations of Arnold ('41), Brown-Sequard ('47), Steinach ('92), Magnus ('99), and Franz (loc. cit.) we know that the excised iris of certain elasmobranchs (Acanthias), teleosts (Anguilla) and amphibians (Rana) responds to illumination by a contraction of the sphincter pupillae.

Hertel ('07), first succeeded in demonstrating a direct response to stimulation by light in the chromatophores of the cephalopods Sepiola, Octopus and especially Loligo. In this case the reactions of the chromatophores are due to contractions and relaxations of the radially arranged smooth muscles. The active phase is here the expanded one, elicited by the contracting radial muscles i.e., it is the reciprocal of the condition in the melanophores of lower vertebrates. Hertel found that ultra-violet light of 280 $\mu\mu$ produced an almost instantaneous local expansion of all chromatophores. Blue rays of 440 $\mu\mu$ and yellow rays of 558 $\mu\mu$ of equal intensity also gave a distinct, but somewhat slower expansion.

I have shown (loc. cit.) that the expanded melanophores of Fundulus respond to ultra-violet light (280 $\mu\mu$) by a rapid and reversible contraction. I was unable however, in a series of trials with different regions and intensities of the visible spectrum to obtain contractions of Fundulus melanophores. Following practically the same technique as in my experiments with Fundulus, Laurens ('15) has recently reported contractions by ultra-violet light in expanded melanophores in pieces of the skin of Amblystoma larvae. Hertel (loc. cit.) had previously found that the melanophores of Triton larvae responded to ultra-violet, blue and yellow rays by contracting. He used the same wave lengths and intensities as in his experiments with cephalopods (vide supra). In this case, as in so many of the older experiments, the presence of a complete nerve mechanism and blood supply presents the possibility of secondary complications. In the case of Fundulus and Amblystoma, however, I believe the results to be free from this objection.

A direct response to light has not thus far been demonstrated in the melanophores of reptiles, though as Fuchs (loc. cit.) has emphasized, it will in all probability be found to exist.

We may now summarize the foregoing observations as follows; 1) in certain species of fish and amphibians the sphincter pupillae contracts in response to direct stimulation by light; 2) in certain other species of fish and amphibians the melanophores also respond to direct stimulation by light by contracting.

III. Effect of Electrical Stimulation

Induction currents of sufficient intensity and duration produce contractions in smooth muscle. Such contractions may be seen in strips of frog's stomach prepared according to Meigs ('12), or in preparations of the digestive tube of several species of teleosts.[5] Beer ('94, '98) observed that the sphincter pupillae of the eel and the frog (Rana) contracted when stimulated electrically. The radial muscles of the chromatophores of cephalopods also respond to electrical stimulation by contracting (Brücke '52, Keller '73, Fredericq '78, Klemensiewicz '78, Pouchet '76, Krukenberg '80, Phisalix '92, Steinach '01, Hofman '07, '10, and Fuchs '10). Klemensiewicz (loc. cit.) records similar contractions in isolated pieces of the skin of Loligo.

Lode (loc. cit.) first showed that in excised pieces of the skin of the trout the melanophores contracted upon being stimulated by an induction current. In my own experiments with Fundulus the melanophores invariably contracted, reversibly, when proper strength and duration of the current and salt concentration of the mounting medium were selected.

Winkler ('10) found that the melanophores of Rana esculenta and Hyla arborea contracted when directly stimulated by an induction current. Laurens (loc. cit.) has recently verified this observation in large larvae of Amblystoma opacum. He finds also that: "When various portions of the body are cut out and directly stimulated either with the central nervous system intact or destroyed, a slight contraction of the melanophores is usually induced" (p. 610).

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[5] Unpublished observations made upon the stomach muscle of Stenotomus, Tautoga, Centropristes and Fundulus.

In the case of the reptilian melanophores Bert ('75) and Krukenberg ('80) were able to corroborate the original observation of Brücke ('52) who found that direct faradic stimulation of excised bits of dark skin in the chameleon produced a lightening, i.e., a contraction of the melanophores.

Thus the responses of several types of smooth muscle, as well as of the melanophores, in representatives of all three groups of lower vertebrates show a satisfactory agreement in their contraction to faradic stimulation.

IV. Effect of Mechanical Stimulation

By gently pinching or stretching excised pieces of frog or fish[3] stomach and oesophagus, powerful contractions may be induced, which are reversible provided the stimulus has not been too violent. Precisely the same reaction follows a similar treatment of portions of the skin of Loligo; the chromatophores expand widely. I have verified this observation by Klemensiewicz loc. cit. and all of the more recent investigations upon cephalopod chromatophores record a similar phenomenon in Loligo and other species.

Fuchs (loc. cit) has called attention to the objection against the expansion observed by many of the older investigators after the surfaces of varios teleosts had been more or less violently 'stroked' with a needle. He says (p. 1432): "Aus allen Beobachtungen geht unstreitig hervor, dass einwandfreic Beobachtungen über die direkte mechanische Reizbarkeit der Fischchromatophoren nicht vorliegen." I have shown (loc. cit.) that by selecting scales from the lateral portion of Fundulus where the melanophores are relatively far apart, it is possible to stimulate a single melanophore repeatedly by exerting a gentle pressure with a fine, fire-polished glass needle. Great care must be exercised not to rupture the delicate cells, for by so doing, the melanin granules are scattered and produce the effect of an 'expansion' recorded by the older observers. In this case it is certain that mechanical stimulation (gentle pressure) produces a reversible contraction of the melanophores.

As a result of local pressure or pinching with forceps, and along the margins of an incision, the melanophores in the frog contract (v. Wittich '54, '71, Hering l. c., Lister '58 a, '58 b, Fuchs '06, Êtérnod and Robert '08). The darkening of the skin in Polypedates Reinwardtii, "nach leichtem Kratzen mit einer Nadel," observed by Siedlecki ('09), is doubtless another case of ruptured melanophores.[4]

The above observations warrant the conclusion that in the radial smooth muscles of the chromatophores of cephalopods, in the smooth muscle of the digestive tract in certain teleosts and amphibia, as well as in the melanophores of these two vertebrate groups, mechanical stimulation (gentle pressure) is followed by a reversible contraction.

V. *Effects of Chemical Stimulation*

A great many experiments have been carried out upon the effects of various widely differing chemical stimuli upon the melanophores of vertebrates. The method of procedure has been, in most cases, as follows; 1) the substances were brought directly upon the skin of the normal or operated (pithed, etc.) animal, the color-change being considered the criterion of the action of the chemicals; 2) the substances were injected into the circulation, the body cavity, or subcutaneously, the color-change again serving as an indicator of chemical stimulation; 3) the substances were added to the water of the environment, in aquatic forms. In relatively few instances have excised pieces of skin been immersed in the fluid to be tested. As I have repeatedly emphasized, this is the only satisfactory method of determining the direct effect of any stimulus upon the melanophores, for it is only in this way that the circulation and all central nervous control may be simultaneously and effectively eliminated. The objection may be raised that even in excised pieces of the animal it is impossible to destroy the ultimate nerve terminations, hence we are unable to state with certainty that the effect is a direct one and not transmitted through the cut stumps of the sympathetic nerves which remain in such a preparation. I have elsewhere (loc. cit.) adduced evidence to show that this objection is certainly invalid in the case of the isolated melanophores of the scales of Fundulus. I shall limit the following discussion of the reactions of melanophores to chemical stimuli to cases which are as nearly as possible comparable to the chemical stimulation of isolated smooth muscle.

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[4] Observations upon the response to pressure in reptilian melanophores are contradictory and unsatisfactory. Most of the experiments have been made with living animals and the few trials with excised bits of skin were all carried out without regard for the possibility of a darkening resulting from the destruction of the melanophores (Milne-Edwards '34 a, '34 b, Brücke '52, Carlton '04).

A. *Inorganic Substances.* Schultz ('97) observed that distilled water acted as a weak contracting stimulus in opened ring preparations of the stomach of the frog. Meigs ('10) has shown that distilled water gives a typical curve of contraction in preparations of longitudinal strips of the frog's stomach.

I have found (loc. cit.) that the melanophores of Fundulus slowly contract when brought from the living dark fish or from 0.1 N NaCl to distilled water (fig. 1).

Schultz (l. c.) noted a relaxation and swelling of the smooth muscle of the frog's stomach in 10 per cent NaCl solution. Meigs (loc. cit.) has shown that in solutions of KCl, the stomach muscle of the frog (R. pipiens) slowly contracts and loses weight while in NaCl it elongates and aborbs water.[5] I have observed that the chromatophores in pieces of the mantle of Loligo expand immediately upon being immersed in 0.1 N KCl solution. This is obviously due to a contraction of the radial smooth muscles. Furthermore, provided the exposure to KCl has not been too long, when such chromatophores are returned to 0.1 N NaCl or better, Ringer solution, they contract again, the radial muscles are relaxed.

The melanophores of all the species of teleosts with which I have experimented, showed a contraction in 0.1 N KCl solution and an expansion or relaxation in 0.1 N NaCl, dilute sea-water, or Ringer's solution.[6] The effects of other neutral salts of the alkalies were also studied in considerable detail but, since, so far as I am aware, no parallel experiments have been performed upon smooth muscle, a discussion of these results would be irrelevant. It is, however, of interest that both cations and anions of the neutral salts show the same order of physiological effect, the so-called 'lyotropic order,' as in experiments upon striated muscle (Overton '04 and Scwartz '07) and certain colloids (Hofmeister '91, Pauli '99, Höber '14, Chapter 7).

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[5] Zoethout ('02) found that KCl produced a contraction in the gastrocnemius of the frog and NaCl a relaxation.

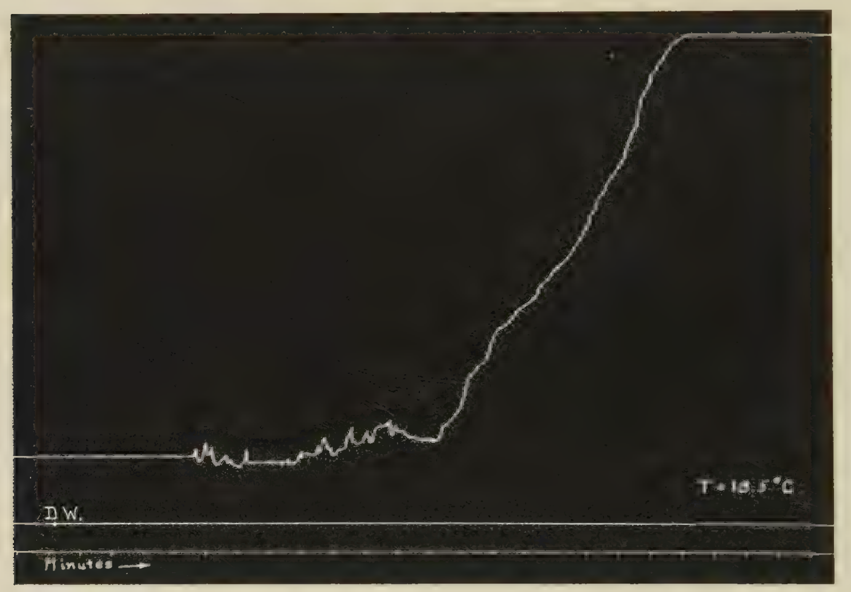

Fig. 1 The slow contraction of a melanophore in distilled water. The upper line represents the movements of the terminal pigment granules in one process of the cell. The lower line indicates time in minutes. At D. W. the cell was immersed in distilled water (from 0.1 N NaCl). The melanophore was completely contracted in twenty minutes. In this case the migration of the pigment granules was followed by means of an ocular micrometer carrying a moveable scale. An empirically selected line of the micrometer scale was kept tangent to the terminal pigment granules by turning the adjusting screw of the ocular. The motion of the screw was transmitted to a set of pulleys and a heart lever and the curve was recorded on a kymograph in the usual way. The actual path of the pigment migration was 0.104 mm. which makes the magnification of the reproduced figure approximately × 534. A detailed description of this apparatus will appear shortly in the American Journal of Physiology.

Recently[6] I have found that the alkaline earths produce a contraction of the melanophores in Fundulus. The time for this contraction in isotonic solutions of the neutral chlorides varies in the order—

$$Ba < Sr < Ca < Mg$$

In strontium, calcium and magnesium chloride, the contractions are all reversible, the time required for complete recovery varying in the reciprocal order—

$$Mg < Ca < Sr$$

The behavior after treatment with 0.1 N $BaCl_2$ for ten minutes is peculiar in that, frequently, upon being returned to 0.1 N NaCl, no sign of recovery appears for as long as thirty minutes. After such a longer or shorter period of quiescence, the peripheral melanophores suddenly expand slightly and contract again almost immediately. After a brief interval of inactivity, a second expansion wave appears at the periphery, but this time the melanophores lying nearer the centre of the scale, which had previously remained contracted, show a slight expansion and those at the periphery expand further than the first time. In this way there is set up a rhythmic expansion and contraction wave which, creeping towards the centre of the scale, gradually comes to include all the melanophores. In the course of an hour after being returned to sodium chloride, all the melanophores of a scale are slowly pulsating. This pulsation may continue at ordinary temperatures for as long as four or five hours, the expansion gradually becoming less complete and frequent and eventually ceasing, the melanophores remaining contracted. In cases where the immersion in $BaCl_2$ has lasted only about five minutes, the pulsations appear upon being returned to NaCl, but

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[6] In scale preparations of Fundulus, the small dermal blood vessels always contain a few corpuscles. When a scale is immersed in a KCl solution these corpuscles begin to move within the vessels and this movement continued approximately as long as the melanophores are contracting. On being returned to NaCl, the melanophores expand and the corpuscles return to their original position or pass beyond it. This movement of the corpuscles may be due to a contraction and relaxation of the walls of the larger blood-vessels.

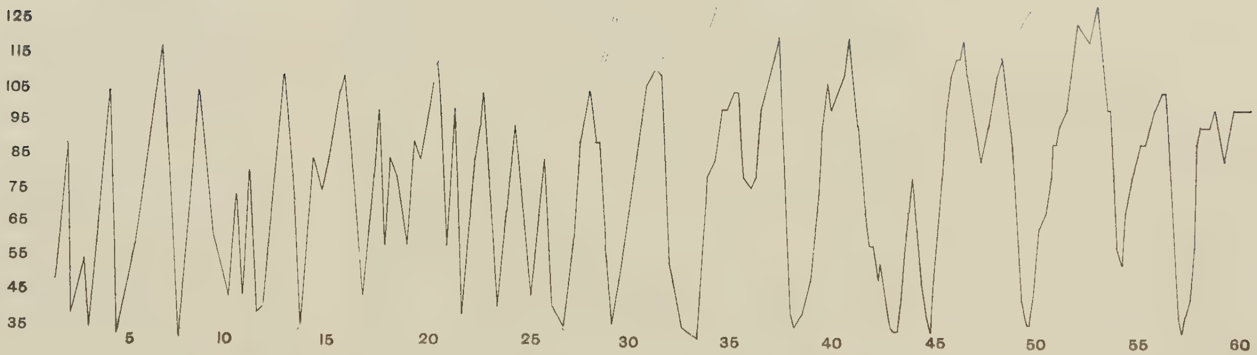

Fig. 2 A typical case of rhythmic pulsation in the melanophore after treating with $BaCl_2$. The curve represents changes in the diameter of a single melanophore in the course of an hour. The maximum diameter of this cell was measured by means of an ocular micrometer at various times. These diameters are plotted against the time; 33 divisions of the micrometer represents complete contraction and 130, extreme expansion. The treatment of this cell was as follows:

Experiment S, 15, 20. June 21, 1915.

7.43 a.m. Scales from an 8.5 cm. female F. heteroclitus were immersed in 0.1 N NaCl.

8.14 a.m. 3 scales to 0.1 N $BaCl_2$.

8.27 a.m. One scale returned to 0.1 N NaCl; temperature 19.4°C.

9.14, 15. First observation of rhythmic contractions.

the contractions between expansions are incomplete, the reduction being in this case in the contractions. The disappearance of the pulsations leaves the melanophores expanded instead of contracted. We may therefore say that the melanophores may recover after having been contracted in $BaCl_2$, provided the time of immersion in the $BaCl_2$ has not been more than about five minutes (fig. 2).

Automatic pulsations or rhythmic contractions occur not uncommonly in several types of smooth muscle. Among the lower vertebrates, for example, Schultz (l. c.) has described this phenomenon in ring preparations of the stomach of the frog and Stiles ('01) has studied it in similar preparations of the frog's oesophagus. An examination of their figures shows that the rapidity and frequency of the contractions is not unlike those occurring in the melanophore after having been treated with $BaCl_2$ and returned to NaCl.

B. *Organic Substances.* Krukenberg ('80) carried out a series of experiments upon the reactions of the chromatophores of Eledona, a cephalopod, to organic substances. He found that 5 per cent ethyl alcohol, ether and chloroform (strength not stated in either case) produced a contraction of the radial smooth muscles i.e. an expansion of the chromatophores. When pieces of the skin were washed or returned to sea-water, the chromatophores contracted and regained their irritability. Klemensiewiecz ('78) observed a lightening of the skin in Loligo and Eledona when subjected to vapors of amyl nitrite. The chromatophores in isolated pieces of skin showed the same reaction. Amyl nitrite is a familiar vaso-dilator. The relaxation of the radial muscles in the chromatophores of cephalopods therefore suggests that this may be a typical response for physiologically widely differing varieties of smooth muscle.

I have exposed the melanophores of Fundulus to alcohol, ether and chloroform vapors. The scales bearing these melanophores were placed upon glass slides and just covered with Ringer's solution. The slides were then placed over Syracuse glasses containing the organic fluid to be tested and both were covered with small, ground-edged, bell jars and sealed with vaseline.

Under these circumstances the melanophores always contracted.[7] Control cells under identical sealed jars, remained expanded.

I have also exposed contracted melanophores of Fundulus in a contracting mixture of 4 parts of 0.1 N NaCl + 1 part 0.1 N KCl to the vapors of amyl nitrite, using the same technique as in the trials with ether and alcohol. All these melanophores expanded promptly whereas control cells in the same contracting mixture, under a second sealed bell-jar, showed no change.

Weak solutions of atropine or atropine sulphate (0.00025 M in 0.1 N NaCl) produce a prompt expansion of the melanophores in Fundulus. The dilating action of this alkaloid upon the pupil is a familiar physiological phenomenon.

Lieben ('06) found that in R. temporaria, subcutaneous injections of adrenalin produced a complete contraction of the melanophores in 15 to 20 minutes. The local application of adrenalin upon the swimming web also produced a contraction. These contractions Lieben believed to be independent of the constricting action of the adrenalin upon the blood vessels. When the melanophores of Fundulus are immersed in solutions of adrenalin hydrochloride of different concentrations[8] they invariably contract. Even at a dilution of one part to one million (in 0.1 N NaCl) a contraction appears, though not always in all the melanophores of a single scale.

From the foregoing observations it appears that certain organic substances which typically produce contractions in smooth muscle, cause a contraction in the melanophores of Fundulus. Reciprocally, certain other organic substances which produce an expansion or relaxation in isolated smooth muscle, produce an expansion in the melanophores of Fundulus.

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[7] The contraction depends, however, upon the amount of organic fluid in the Syracuse dish. Under the conditions of these experiments, 3 to 5 drops produced a contraction. Larger amounts inhibited the contraction and produced a narcosis. This became evident when the cells were removed from the bell jars, for a transient contraction appeared, the effect of the dilute stimulus.

[8] A Parke-Davis preparation of adrenalin hydrochloride 1 : 1000 in physiological salt solution was used as the starting point in these experiments. This was diluted with 0.1 N NaCl to the required concentration.

AN ANALYSIS OF THE CONTRACTION IN THE MELANOPHORE

A structural and functional parallelism between smooth muscle and melanophores can scarcely be considered complete without a comparison of the contractions in the two cases. At first sight they have little in common. Indeed the exact mechanical nature of the contraction in the melanphores is still a disputed question. Certain facts are established beyond any reasonable doubt viz: 1) the proximal and distal pigment migrations invariably occur along fixed paths (Kahn u. Lieben '07, Spaeth '13 b); 2) in cases where living cells have been observed under high magnifications it has appeared that the pigment granules are not merely carried along passively by a streaming or flowing of an extremely liquid sort of protoplasm, but that they exhibit active, independent movements within the cell processes (Ballowitz '13 a and b, Degner '12). Exactly what occurs after the pigment granules have reached the centre of the cell, has not been determined as yet with certainty. Either 1) the cell processes remain in situ or 2) the more fluid protoplasm follows the migrating pigment granules and eventually the entire cell becomes rounded up as in a contracted amoeba. In the latter event it is difficult to imagine how the protoplasmic processes find their way back again to the original contour of the expanded cell. Hooker ('12 and '14) has explained away this difficulty by assuming, from histological preparations, the existence of lymph spaces into which the pseudopodia of the amoeboid melanophore creep or flow. Hooker believes these lymph spaces to be fixed and the melanophore to be a typical amoeboid cell. There are serious objections to this view. 1) Hooker's evidence is adduced from fixed preparations and he has been unable to corroborate his observations upon living adult cells; 2) in the many hundreds of living melanophores that I have observed under high magnifications and the most favorable optical conditions (reflected and transmitted light, dark-field illumination, etc.) I have never been able to detect the secondary migration of the fluid protoplasm, though I have repeatedly watched the same cell in a series of expansions and contractions with this as the sole object; 3) the histological preparations of Ballowitz ('93) show nerve terminations on the processes of the melanophores which could not have any physiological significance if the entire cell were withdrawn from these processes; 4) finally I have repeatedly corroborated in Fundulus, the observation of Ballowitz ('13) concerning the position of the nucleus following a proximal migration of the pigment granules. Not infrequently in an expanded melanophore, the nucleus may be seen to lie far out from the centre in one of the processes. If such a cell be contracted, all the pigment granules accumulate at the centre, but the nucleus remain in its original position in the process. Under favorable conditions it may actually be seen in the contracted cell, lying in the process, with a considerable area of pigment-free cytoplasm between it and the central mass of melanin granules. In any case, upon reexpansion, the nucleus is found practically to have retained its original position. It is difficult to correlate these facts with Hooker's conception.

At present it is, then, impossible to say that the melanophores are certainly not amoeboid cells in the mature condition, though the weight of the evidence appears to be against such a view.

The question now arises as to what a proximal and distal migration of pigment granules within a stellate cell can have in common with a 'contraction' in smooth muscle. A physical-chemical analysis of the melanophore may serve to clarify the comparison.

Considered as a physical-chemical system the melanophore consists essentially of a colloidal suspension of melanin granules (the disperse phase) in a dispersion medium which is itself an exceedingly fluid sort of protoplasm i.e., an emulsoid sol. 'Contraction' of the melanophore consists of an aggregation of the disperse phase of melanin granules and in 'expansion' there is an increased dispersion of the melanin granules. In other words, when the melanophores are stimulated to contract, we observe the first step in a reversible aggregation or coagulation process, i.e., the aggregation of the melanin granules. Is it possible to consider this phenomenon a reversible coagulation such as occurs commonly in emulsoids? The size of the melanin granules is relatively so large (ca. 0.0004 mm.) that we should rather expect an irreversible coagulation as we find among suspensions or suspension colloids. The presence of the fluid protoplasm in which the granules are suspended must here be taken into consideration. It is a well known fact (Bechhold '04) that the disperse phase of a suspension colloid or suspension, adsorbs the particles of the disperse phase of an emulsoid when the two are in 'solution' together. This phenomenon has been termed a 'protection' of the coarser phase; the adsorbed emulsoid is the protecting colloid ('Schutzkolloid' of Bechhold). Under such circumstances, the suspension colloid assumes the character of a true emulsoid, i.e., it is protected against the irreversible coagulative effects of electrolytes, heat, etc. Suspension colloids or suspensions, in the presence of emulsoids, thus become far more stable and plastic systems (Neisser und Friedemann '04). The existence of just such a physical-chemical system within the melanophore is a perfectly demonstrable fact. Its reversibility is equally obvious. Thus far, then, we have but analyzed the physical-chemical conditions within the cell based upon the observed facts.

In the case of an increased dispersion in a two-phase system, there is an increased intimacy of relation between disperse phase and solvent; the system approaches a true solution and we speak of an increased solubility of the disperse phase. Reciprocally, in an aggregation or coagulation process, we have a separation of disperse phase and solvent. There is an accumulation of evidence at hand showing "that during the contraction of smooth muscle there is an exchange of fluid between the cells of the tissue and their surroundings" (Meigs '12, p. 543). It is difficult to imagine how this setting free of fluid can occur in a colloidal system such as a smooth muscle cell, except in connection with a reversible coagulation or aggregation process. In other words, evidence for an exchange of fluid during contraction in both striated and smooth muscle must, at the same time, be considered evidence for a reversible aggregation or coagulation process in the cell colloids. The displacement of fluid is as necessary an accompaniment of the act of contraction as is the coalescence of colloidal particles.[9]

[9] A discussion of the relative merits of the 'swelling' and 'surface-tension' hypotheses of muscle contraction would be irrelevant (Lillie '12).

In the case of the melanophores there is no direct evidence at hand for a loss or exchange of fluid during contraction. There is, however, a visible and reversible colloidal aggregation of melanin granules following a variety of physiological stimuli, all of which elicit contractions in smooth muscle. Similarly, a number of physiological stimuli that produce a relaxation in smooth muscle bring about a dispersion of the pigment granules in the melanophore. Now, from the evidence cited above, we are forced to assume that, during the contraction of smooth muscle there is an invisible aggregation or heaping up of colloidal particles which becomes reversed in relaxation, i.e., when the muscle relaxes, there is an increased dispersion of the invisible colloidal particles. *The aggregation of melanin granules within the melanophore must therefore be considered a visible expression of the colloidal phenomenon that occurs, upon stimulation, in the micro-homogeneous colloidal content of a smooth muscle cell.*

GENERAL APPLICATION OF THE CONCLUSION

Although much of the evidence in the preceding sections has been obtained from experiments with a single species of teleost, Fundulus, there is no valid reason for doubting that the mechanism of the responses in the chromatophores of crustaceans and the melanophores of fish, amphibia and reptiles, is identical.[10] In all these cases the color-changes are brought about by an aggregation and dispersion of pigment granules. Chun (l. c.) has shown that in cephalopods the chromatophores develop from a single smooth muscle cell. We may therefore say that it seems highly probable that color changes in crustaceans and cephalopods, as well as in the three groups of lower vertebrates, are brought about by the physiological responses of specialized smooth muscle cells.

The physiological modification in the case of the melanophore may be compared with that of the electric organ in fish where a striated muscle cell becomes so far modified that the normal motor function disappears completely and the action current, which is normally of relative insignificance, becomes a physiological end in itself and a formidable means of protection. Similarly in the melanophore, the motor function of the smooth muscle cell is lost and there is developed a modified motility, a migration of pigment granules, which may again serve as a means of protection.

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[10] The term 'chromatophores' can not be applied to the case of the lower vertebrates until more is known of the physiology of the xanthophores.

SUMMARY

1) Morphological, embryological and physiological evidence is advanced to prove that the melanophores of fish, amphibia and reptiles are not connective tissue cells as has been tacitly assumed heretofore, but functionally modified smooth muscle cells.

2) In the contraction of the melanophore there is an aggregation of melanin granules which is to be considered the visible counterpart of an aggregation of colloidal particles that occurs during the contraction in smooth, and possibly striated muscle.

3) It seems highly probable that color-changes in crustaceans and cephalopods, as well as in the three groups of lower vertebrates, are brought about by the physiological responses of specialized smooth muscle cells.

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ON THE EFFECT OF REMOVAL OF THE PRONEPHROS OF THE AMPHIBIAN EMBRYO

By Ruth B. Howland
SWEET BRIAR COLLEGE, SWEET BRIAR, VA.

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The following note embodies the results of a series of experiments, performed at Yale University at the suggestion of Dr. R. G. Harrison, on embryos of the spotted salamander, Amblystoma punctatum. The particular problems in mind were first, to determine whether the head kidney or pronephros is a functioning organ necessary to the life of the embryo, and second, to investigate the correlation of the development of this organ with that of other components of the excretory system.

The embryos used for the experiments were nearly of a uniform age, varying slightly in size and degree of development from the condition in which the first loop of the pronephric tubules appears as a slight, ventrally directed curve of the duct, to the stage in which the two funnels, together with the first loop, appear as a broadened Y. (Fig 1.) The tail-bud was clearly defined, and the pronephric swelling distinctly visible. In all cases, however, embryos were used before contraction of the body muscles began, for contraction not only hindered the operation, but often tore open the wound after successful removal of the kidney.

Two methods were employed in removing the pronephroi. In the first, three straight cuts were made, one beneath and one along each side of the pronephric prominence. The flat of skin thus defined was lifted up and the organ removed from below. In the second and more satisfactory method, a single incision was made dorsal to or immediately over the thickening, the needle inserted, and the tubule raised upward from the ventral side and excised.

Conditions ensuing upon the removal of the head kidney of both sides in Amblystoma larvae show clearly that these organs are necessary to the life of the embryo, although the presence of one pronephros suffices to keep the organism alive and in a healthy condition. All embryos from which both head kidneys had been excised died within eight to twelve days, evidencing during that interval symptoms of weakened heart-action—oedema and effusion into the pericardial and abdominal cavities—presumably brought about by uremic poisoning. Pricking the body wall to relieve the dropsical condition was resorted to in many cases from five to seven days after operating, but it proved ineffective.

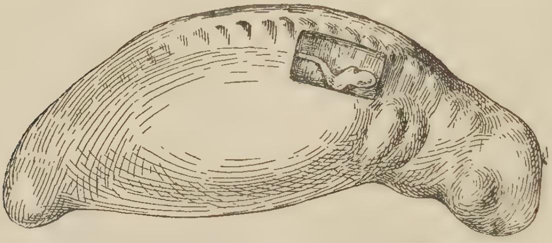

FIG. 1.—AMBLYSTOMA EMBRYO IN THE OPERATING STAGE, SHOWING THE PRONEPHROS EXPOSED AFTER REMOVAL OF ECTODERM. × 13.

The pronephros remaining after the removal of one head kidney evidently takes over the function of excretion usually performed by the two organs, and, concomitant with the increased physiological activity, presents marked morphological changes. The size of the organ which functions alone is greatly increased, indicating the occurrence of compensatory hypertrophy. In the normal pronephros, the walls of the tubules are thick, and consist of cuboidal cells, the central ends of which often bulge out into the narrow lumen. The hypertrophied tubules are thin walled, the cells flattened as is the case in normal larvae of greater age, and the lumen accordingly is nearly twice that of the unoperated specimen. To determine the nature and extent of the change brought about in the functioning pronephros through the removal of the organ on one side, wax models of this organ (× 200) of an operated specimen and of a normal embryo were constructed. The control was chosen from a large number of normal embryos which, on sectioning, showed the several organs (retina, lens of eye, digestive tract, etc.) to be in a stage of development identical with that in the operated individual. On comparison of the models, the evidence of hypertrophy shown in microscopic examination was strengthened. The model of the operated individual not only showed a considerable increase in length of the coiled tubule as contrasted with the control, but by its size indicated a great increase in volume. The length of the tubules making up the models was determined by taking the average of five measurements. The larger model measures 188 cm. (× 200), the smaller model 155 cm. (× 200), showing an increase of 21 per cent over the normal conditions. Difference in weight also indicates hypertrophy, the normal model weighing 62.9 gr. as contrasted with the larger, which has a weight of 115.9 gr.

The formation of the pronephric glomeruli under normal conditions as outgrowths from the aorta toward the newly forming kidneys suggested the possibility that in the operated specimens the glomeruli of one side might be lacking. The removal of one pronephros has, however, no effect on this development, for in all of the operated embryos both glomeruli were present. The glomerulus on the operated side is not as uniform in size and shape as the normally functioning one, its form evidently being largely determined by the width of the coelomic cavity in this region. The outer layer of the glomerular capillaries and the epithelial lining of the body wall often coalesce. Absence of the pronephros enlarges the cavity on that side, and the glomerulus is then found bridging this cavity as a long strand instead of appearing as a more compact tuft of capillaries.

In the removal of the embryonic pronephros, as much of the tissue was scraped out as seemed possible without disturbing the Anlage of the aorta. In spite of this fact, a large number of the operated embryos possessed well developed anterior and posterior nephrostomes and portions of the funnels, indicating that these structures are probably regenerated from the adjacent coelomic epithelium. Of the sixteen embryos examined, twelve showed well formed anterior funnels and nine had posterior funnels. In one instance the anterior funnel was of a peculiar double form, suggesting the normal condition in those amphibia with three pronephric openings.

The effect of excision of one pronephros on the formation of the segmental duct on the operated side varied widely. The process of development of the non-functioning ducts is carried on only to a limited extent. In the embryos examined, every gradation of development was found from a condition in which the lumen, though small and flattened dorsoventrally, appears throughout the entire length, to a condition where only the occasional presence of a few degenerating cells indicates the location of the atrophied duct.

Increased activity of a single kidney also has a definite effect on the segmental duct of that side. Cross sections of the duct of an individual with unilateral operation, when compared with either of the ducts of a normal larva of the same stage, show a marked increase in diameter.

The mesonephroi of both sides develop normally, at least in the early stages, even after excision of one pronephros. Non-development of the segmental duct in one instance left the mesonephric tubules disconnected on that side, and with no outlet for excretory products. No specimens have yet been kept a sufficient length of time to determine the ultimate outcome of this abnormal condition.

In brief then, the following conclusions may be drawn.

1. Removal of both pronephroi in Amblystoma larvae induces conditions leading to oedema and subsequent death, though the presence of one head kidney is sufficient to keep the embryo in a condition of health.
2. Excision of one head kidney brings about an increase in size in the remaining organ, and also in the diameter of the segmental duct on that side.
3. Removal of one pronephros has no essential effect on the development of the pronephric glomerulus of that side, but the segmental duct appears in varying stages of atrophy.
4. Anterior and posterior nephrostomes may regenerate from the coelomic epithelium.
5. Early developmental stages of the mesonephros are normal, even after excision of one head kidney.

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THE VITAL EQUILIBRIUM

FOLLOWING the suggestion of Nernst[1] that varying degrees of permeability of the plasma membrane might be due to a selective solubility of certain of its components, Overton established his lipoid theory. The most serious objection to Overton's theory is that, whereas it accounts most satisfactorily for the permeability of the cell for substances which normally play no part in the cell metabolism, it entirely fails to explain the penetration of sugars, salts and amino-acids, which must constitute an essential part of the cell income. Loeb[2] long ago emphasized the importance of the state of aggregation of the surface colloids as one factor influencing the conditions of permeability. This suggestion was made in connection with his experiments upon the effects of pure solutions of NaCl and combinations of NaCl and polyvalent ions on the eggs of *Fundulus.* Subsequent experiments by Loeb, Höber, Lillie and a host of others, have established beyond a doubt the existence of a physical-chemical relation between the state of aggregation of the cell colloids and the permeability of the cell. A precise and universal statement of the exact nature of this relation has never been made. In the following paper we shall attempt an analysis of the conditions determining the viscosity of cell surfaces and their importance; (1) in producing changes in permeability and (2) in "antagonisms." It appears that the metabolic activities of cells, in so far as they involve an interchange of material through the surface layer, depends upon the shifting of a *surface-solution equilibrium.* Since an interchange of material eventually becomes essential for the continuation of any living system, we have called this solution equilibrium the "Vital Equilibrium."

If we examine a series of two-phase systems beginning with a coarse suspension and extending through fine suspensions, colloidal suspensions, colloidal solutions, hydrophilous colloidal the "molecular disperse" systems of Ostwald and ionically disperse systems such as dilute solutions of electrolytes like NaCl, we observe two striking changes: first, an increased subdivision of the disperse phase and, second, an increased intimacy of relation between disperse phase and solvent, a necessary result of the enormously developed surface in the former.[3] We find, furthermore, that in any of these systems there always exists an equilibrium between disperse phase and solvent. The opposing forces are in the direction of an increased aggregation and dispersion, respectively; we may therefore speak of an aggregation equilibrium. This equilibrium is shifted by the addition of solutions of any substance, organic or inorganic, by heat, ultraviolet light, etc. For example, if we add $CaCl_2$ to the negative suspension colloid As_2S_3, a precipitation occurs, *i. e.,* there is an increased aggregation of the disperse phase. Reciprocally, small quantities of 0.1 N $CaCl_2$ will clear an opaque colloidal solution of egg-white. There is an increased dispersion and the system becomes more like a true solution. Now the limits of the above series are total insolubility and complete solubility. Any change in the direction of increased dispersion means a change in the direction of a true solution, *i. e.*, an increased solubility. No sharp limits occur between true solutions and colloidal solutions. A solution of cane sugar, for example, though a molecular disperse system, certainly represents a lesser degree of dispersion than any solution of an electrolyte. Again, when two salt solutions having a common ion are combined, there appears the familiar phenomenon of association or decreased dispersion, an equilibrium shift in the direction of greater aggregation, in this case from ionic to molecular dispersion. We may therefore legitimately dispense with the term "aggregation equilibrium" and, even though we are dealing with colloidal systems, substitute the more familiar "solution equilibrium."

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[1] Nernst, W., '90, *Zeitschr. f. physikal. Chem.,* 6, 37.

[2] Loeb, J., '01, *Pflügers Arch.,* 88, 68; '02, *Amer. Jour. of Physiol.,* 6, 411.

[3] Höber, R., '14, *Physikal. Chemie d. Zelle u. d. Gewebe.* 4 Aufl., Kap. 7, p. 305 ff.

The hydrophilous colloids which are of particular interest to physiologists are peculiarly susceptible to slight changes in hydrogen ion concentration. Here the changes in aggregation are reversible to a far greater degree than in the colloids lower in the scale. The limits of reversibility of the solution equilibrium may be said to include a far greater range of aggregation states than in the colloids of the lower classes.

An examination of the experimental data[4] shows that for a number of different hydrophilous colloids the following anion order of dispersion obtains:

$$SO_4 < \text{Tartrate} < \text{Citrate} < \text{Acetate} < Cl < ClO_3 < NO_3 < Br < I < SCN.$$

The most indifferent region lies between acetate and chloride; SO_4 has the greatest tendency towards aggregation, while SCN produces maximum dispersion.[5] In many cases, especially in precipitation experiments, the addition of the electrolyte may have no visible effect upon the colloid. When this occurs the changed equilibrium may be detected by a viscosity determination, one of the most accessible methods for tracing changes in the state of colloidal aggregation.

The viscosity of colloids or its reciprocal, fluidity, shows peculiar variations with different degrees of dispersion. When the dispersion is greatest, *i. e.*, when the disperse phase is in "solution," we find that the fluidity is also at a maximum. An increased aggregation means a decreased fluidity which, however, continues only to the point at which the disperse phase begins to separate out from the dispersion medium as a suspension colloid or suspension. When this point is attained, the fluidity is suddenly reversed and approaches more and more that of the pure dispersion medium. Now whether the precipitation of the disperse phase is brought about by the action of electrolytes or, for example, by elevated temperature (heat coagulation) the physical-chemical effect upon the fluidity is the same (Fig. 1, *B*).

Since all substances have an influence one way or the other upon the solution equilibrium of a colloidal system, we may, theoretically, divide them into two groups; (1) those favoring solubility of the disperse phase (increased dispersion, increased fluidity) and (2) those favoring insolubility (aggregation, precipitation, coagulation, initial increased viscosity).

Turning now to the conditions of the colloids especially at the surfaces of cells, we find, in some cases, sharply differentiated membranes. In many animal cells such membranes are not demonstrable, but for our present discussion this is of little moment, since we are concerned with a colloidal boundary which must exist at the surface of every cell. In experimental studies upon single cells as, for example, animal eggs, variations in the constitution of the environmental medium produce profound changes in the cell. Whatever changes may occur within the cell as a result of such variations, it is certain that these changes are secondary to an initial or primary effect at the cell surface. Liquefying agents are believed to produce an increase in cell permeability.[6] *Arbacia* eggs, for example, when treated with solutions of sodium or potassium thiocyanate, begin to lose their pigment after two or three minutes. This is to be regarded as an expression of an increase in the normal permeability of the cell surface.

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[4] Hofmeister, F., '91, *Arch. exper. Pathol. u. Pharmakol.*, 28, 210; Höber, R., '07, *Hofmeisters Beitr.*, 11, 35; Porges u. Neubauer, '07, *Biochem. Zeitschr.*, 7, 152; Hardy, '05, *Jour. of Physiol.*, 33, 251.

[5] By a sufficient increase in the hydrogen ion concentration the anion order may be *completely inverted.* Thus the effect of an alkali salt upon the state of aggregation of any hydrophilous colloid depends directly upon the hydrogen ion concentration. Posternak, '01, *Ann. Inst. Pasteur*, 15, 85; Pauli, W., '03, *Hofmeisters Beitr.*, 5, 27; Höber, R., '07, *ibid.*, 11, 35.

It has been shown for a number of physiological objects[7] that the deleterious action (liquefying action) of neutral alkalin salts decreases from SCN to Cl in an order corresponding satisfactorily with the Hofmeister series. In these experiments, when the solutions of the salts are brought into contact with the cell surface, the degree of dispersion of the surface colloids must be increased. The dispersion is greatest in solutions of thiocyanates and least in chlorides. A physico-chemical expression of this increased dispersion is the increase in the fluidity of the cell surface. Now, since the speed of diffusion of ions and molecules through any fluid medium depends upon the viscosity of that medium,[8] it is clear that an increased fluidity of the cell surface involves a facilitation of diffusion of soluble substances from either side of the cell surface. In other words, by a liquefying action at the surface, the permeability of the cell is increased and diffusion in both directions across the surface is facilitated.

Since there is this very definite correlation between liquefaction (dispersion) and increased permeability, it is obvious that, in the normal condition of the cell, we must have a greater aggregation of the surface colloids than during liquefaction. Bearing in mind the fact that pure solutions of all substances affect the solution equilibrium of colloids one way or the other, we should expect, *a priori*, to find certain agents producing an increased aggregation of the surface colloids of the cell; we should expect to find true solutions of electrolytes or non-electrolytes which, when brought into contact with the cell surface, would reduce the degree of dispersion of the surface colloids. This process is not, however, a simple reciprocal of liquefaction. A *slight* increase in the aggregation of the surface colloids would involve a rise in the viscosity of the cell surface and, if an equilibrium were established, the speed of diffusion of ions and molecules across the cell surface would thus be reduced. As we noted above, the speed of diffusion of any substance across the cell surface is one index of the degree of permeability of the cell for that substance. Hence we may say that with an increase of viscosity at the surface, the permeability of the cell would be decreased. If now the concentration of the agent that is responsible for the increased aggregation at the cell surface were still further increased, there would be an additional increase in the viscosity of the surface. But, as we have already stated, the viscosity of colloids is sharply limited by the state of aggregation of the disperse phase. When the precipitating disperse phase begins to separate out from the dispersion medium, the viscosity suddenly decreases. Similarly, when at the surface of the cell the disperse phase begins to separate from the dispersion medium, *the fluidity of the cell surface* must rise abruptly. The sharp increase in fluidity would obviously involve a sudden removal of the barrier to diffusion for ions and molecules and they would pass the cell surface more rapidly.[9]

From the above considerations it seems, therefore, that the permeability of a cell may be increased either (1) by bringing into contact with the surface a solution of some liquefying agent like a thiocyanate, *i. e.*, some agent that increases the solubility (degree of dispersion) of the surface colloids and the fluidity of the surface, or (2) by bringing into contact with the surface a solution containing an excess of some deliquefying or precipitating agent like $CaCl_2$ which, by increasing the state of aggregation of the surface colloids eventually separates disperse phases from solvent, the fluidity of the cell surface approaching that of the pure dispersion medium.

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[6] Lillie, R. S., '13, *Jour. of Morphol.*, 22, 695.

[7] Schwarz, C., *Pflügers Arch.*, 117, 161; Lillie, R. S., '10, *Amer. Jour. of Physiol.*, 26, 106; Spaeth, R. A., '13, *Jour. of Exper. Zool.*, 15, 527.

[8] In the case of water-swollen gels, the speed of diffusion of crystalloids is approximately the same as in pure water, but it diminishes rapidly when the water content falls below a certain value. Bechhold u. Ziegler, '06, *Zeitschr. f. physik. Chemie*, 56, 105.

[9] Ostwald, Wolfg., '11, *Grundriss d. Kolloidchemie*, 2 Aufl., p. 307.

The normal influences at the cell boundary must have a considerable aggregating effect upon the surface colloids since we do not normally find the cell pigments or other constituents diffusing outwards. Osterhout,[10] furthermore, has shown conclusively that certain electrolytes increase the electrical resistance of a cylinder of *Laminaria* discs. This is to be considered an expression of decreased permeability. These electrolytes ($MgCl_2$, $CaCl_2$, HCl, $La_2(NO_3)_6$) all have, in certain concentrations, a distinct coagulative or dehydrating effect upon a variety of colloids.[11] The effect of $CaCl_2$ is of particular interest since at first it increases the resistance. After a time, however, the resistance again decreases, finally falling below the initial value. This is precisely what we should expect if the effect of the $CaCl_2$ were upon the viscosity of the surface colloids. The theoretical correlation between increased dispersion and increased permeability, as well as that between increased aggregation and initial decreased permeability, is thus actually substantiated by experiment.

Permeability studies upon living cells have brought out one very striking and at first sight anomalous, fact, viz., for many substances which are not concerned with the normal metabolic processes of the cell, the cell surface is readily permeable, whereas for sugars, salts, amino acids, etc., which must constitute a large proportion of its nutritive material, it is nearly or quite impermeable. The latter substances normally occur, however, within the cell, which forces us to assume that at some previous period the surface must have permitted their passage to the cell interior. This passage to the interior of the cell could have been accomplished only under conditions of increased permeability. Now the cell content is obviously not to be regarded as permanent and fixed and we must account for a mechanism of metabolic interchange. Such a mechanism is to be sought in some type of physical-chemical equilibrium that permits the permeability above and below a norm to vary reversibly within definite limits. We have pointed out above that there is in every colloidal system an aggregation or solution equilibrium between disperse phase and dispersion medium. The colloids at the surface of the cell are no exception to this rule. The continued action of a liquefying agent at the cell surface produces a marked increase in permeability and eventually death by irreversible liquefaction. On the other hand, a coagulating agent, *i. e.*, an agent that increases the aggregation of the disperse phases of the surface colloids, produces *at first* a decrease in permeability, but, if the action be sufficiently prolonged, the disperse phases separate out from the dispersion medium and death follows as a result of surface coagulation. Once the disperse phases have begun to separate from the dispersion medium, the fluidity of the cell surface approaches that of the pure dispersion medium which obviously involves a tremendous increase in permeability. Thus cell death, whether by irreversible surface liquefaction or by irreversible surface coagulation, invariably involves an increase in the permeability of the cell. The term "cytolysis" has been loosely applied to cover both cases, though from a physical-chemical standpoint we are dealing with antithetical processes.

The degree of aggregation of the surface colloids, *i. e.*, the degree of intimacy of relation between disperse phases and solvent appears, upon last analysis, to be the critical condition upon which depends the continuation of the cell as a living system. The degree of aggregation of the disperse phases at the surface of the cell is directly dependent upon their solubility in the dispersion medium. This solubility is determined (1) by the concentration, nature and number of electrolytes or organic substances occurring in the liquid phase, and (2) by the temperature of the whole system. We may, therefore, say: (*A*) at the surface of every cell there is a solution equilibrium, a *vital equilibrium*, between disperse phases and solvent; (*B*) the permeability of the cell is determined by the maintenance or shifting of the vital equilibrium.

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[10] Osterhout, W. J. V., '15, SCIENCE, 41, 255 for a summary of results.

[11] Mines, G., '10, *Jour. of Physiol.*, 40, 327; '11, *ibid.*, 42, 309; Höber, R., u. Spaeth, R. A., '14, *Pflügers Arch.*, 159, 433.

We may now summarize the foregoing conclusions as follows:

1. In the limiting colloidal system of every cell, whether in the form of a differentiated membrane or not, there exists an equilibrium between disperse phases and dispersion medium.

2. A shifting of this equilibrium in the direction of greater dispersion causes an increased permeability of the cell surface, since the fluidity of the system is increased, the viscosity of the surface is lowered, and a more rapid diffusion occurs across the surface both into and out of the cell.

3. A slight shift of this equilibrium in the direction of increased aggregation involves a solidifying action at the surface, an increased viscosity, a slower rate of diffusion across the surface and a consequent decrease in permeability.

4. A considerable shift of this surface equilibrium in the direction of increased aggregation (insolubility of the surface colloids) involves a decrease in the degree of intimacy between disperse phases and solvent; the fluidity is suddenly increased and diffusion across the surface is correspondingly facilitated.

5. The critical condition of any cell surface, upon which eventually depends the continuation of the cell as a living system, is the state of aggregation of its surface colloids, *i. e.*, the relation of disperse phases to dispersion medium. We may, therefore, speak of a solution equilibrium, a *vital equilibrium* at every cell surface, reversible within definite limits, the overstepping of which produces death by surface liquefaction, on the one hand, or by surface coagulation, on the other.

We have thus far considered the cases involving the effects of single electrolytes upon the surface colloids of cells. We shall now briefly consider the physiological effects (1) of combinations of electrolytes and (2) of elevation of temperature.

In any combination of electrolytes it is clear that if it were possible exactly to compensate the dispersion effect of one constituent or group of constituents by the aggregation effect of another, the solution equilibrium of the surface colloids of a cell exposed to such a combination would remain unchanged. Stated in physiological terms, if we could compensate coagulative and liquefactive forces at the cell surface, the vital equilibrium would remain normal and we should obtain no injurious effect. Compensating effects of this sort are actually realized in solutions like those of Ringer and Locke, or in sea water. We have here a number of combined chemical stimuli which, when acting singly, produce distinct liquefactive or coagulative effects upon living cells, but which, in combination, are relatively harmless. According to the conception of a solution equilibrium at the cell surface, the non-injurious effects of compensated solutions of two or more constituents are to be referred to the failure of these solutions markedly to increase or decrease the solubility of the colloidal disperse phases. Such phenomena of physiological compensation have been collectively termed "antagonisms."

That there is some physical-chemical principle behind all "antagonisms" is strongly suggested (1) by the appearance of the compensation phenomenon between such widely unrelated chemical substances[12] and (2) by the compensation that appears between liquefying agents and elevated temperature. This last case seems of such importance as to warrant a detailed consideration.

If a colloidal solution of egg-white be gradually heated to 35°–40° C. it becomes slightly less translucent, *i. e.*, there is an increased dispersion. A viscosity measurement[13] shows that an increase in fluidity continues uniformly up to about 57.5° C. At this point there is a sharp reversal of the reaction and the viscosity rises rapidly to about 60.0° C., the coagulation point (Fig. 1, B). Owing to this peculiar property of coagulation, which is, physically, an increase in the state of aggregation, whatever may be the nature of the chemical processes involved, we have here opposite effects produced by a slight and considerable increase in temperature, respectively; the effect of heat may be either liquefactive[14] or coagulative. We should expect, *a priori*, that by adding a powerful liquefying agent to an hydrophilous colloid, the coagulative effect of heat might be overcome wholly or in part, since this would introduce a dispersion factor into the equilibrium. This actually occurs as Pauli[15] and Pauli and Handovsky have shown. Pauli found that upon adding neutral thiocyanates, which are powerful liquefying agents, to egg-albumin, it could not be coagulated even at the boiling point of the mixture.

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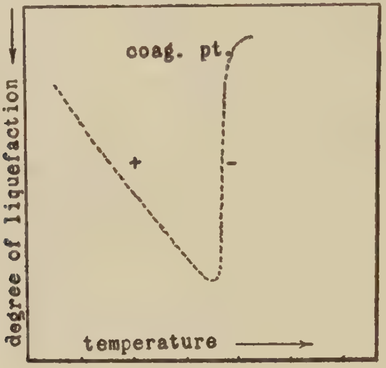
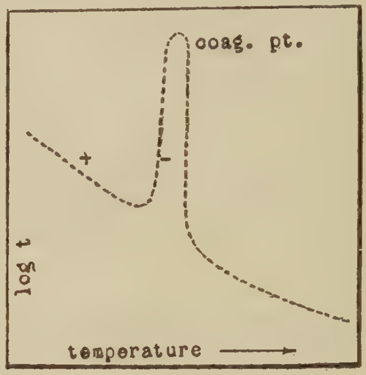
[12] For example "antagonisms" have appeared between various alkaloids such as atropine and eserine, nicotine and curare, between alkaloids and salts as atropine and $CaCl_2$ and $MgCl_2$ and between such salts as KCl and cobalt hexamine chloride (Höber u. Spaeth, *loc. cit.*).

[13] Ostwald, Wo., '13, *Koll. Zeitschr.*, 12, 213.



A.

FIG. 1, *A*. An empirical curve representing the liquefying action of atropine upon the melanophores of *Fundulus* at different temperatures. The points are relative. Thus at 22° C. there is more liquefaction than at 10° C., but less than at 36° C. At 37° C. there is again less liquefaction than at 22° C., but more than at 5° C. Up to 36° C. the temperature coefficient of liquefaction is therefore positive, while beyond 36° C. for a few degrees, it becomes negative.



B.

FIG. 1, *B*. Ostwald's curve showing the effect of elevated temperature upon the viscosity of egg-white. Here the temperature coefficient of liquefaction is positive up to about 57.5° C., while beyond this point to about 60° C. it becomes negative.

A comparison of the two curves shows that above and below a critical point in each system (36° C. and 57.5° C.) the temperature effects are antithetical.

Four years ago I observed that the liquefying effect of atropine or atropine sulphate upon the melanophores of *Fundulus* could be distinctly reduced by sufficiently elevating the temperature of the solution. Recently[16] I have found that for temperatures up to approximately 36° C., atropine shows a normal positive temperature coefficient, *i. e.*, the liquefying effect increases with a rise in temperature. If, however, we expose contracted melanophores to identical solutions of atropine at 22° C. and at 37° C. for a period of five minutes, the cells from the warm solution show distinctly less liquefaction than those at room temperature. That the cell colloids are not coagulated by the higher temperature is shown by the activity of the cell upon being returned to NaCl or KCl solutions. Thus in this case, for a few degrees, between 36° C. and the elevated coagulation point of the cell protoplasm ($< 43°$ C.) the temperature coefficient of liquefaction for atropine becomes negative (Fig. 1, A). From the foregoing considerations we should expect an elevation in temperature to increase the solubility of the disperse phases at the surface of the melanophore.[17] We have, in addition, the liquefying effect of the atropine. Hence we have here the combined liquefying effect of atropine and elevated temperature, *i. e.,* two forces tending to drive the disperse phases of the cell surface into solution and to increase their degree of dispersion. Beyond 36° C., however, further increase in temperature tends to initiate the first steps in the process of heat coagulation involving a decrease in the state of aggregation of the surface colloids. The inhibition of the atropine effect above 36° C. is, therefore, to be interpreted as due to an elevation in the viscosity of the surface colloids which retards the diffusion of the alkaloid into the cell. Bearing in mind these antithetical physical effects of low and high temperatures, it appears that the experimental data both in the case of colloidal solutions of egg-white and in that of living cells (melanophores) comply well with the theory.[18] A liquefying agent in proper concentration may prevent heat coagulation and, reciprocally, a sufficient elevation in temperature may protect the system against liquefaction.

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[14] Lillie, R. S., '15, *Biol. Bulletin*, 28, 260.

[15] Pauli, W., '99, *Pflügers Arch.*, 78, 35; Pauli u. Handovsky, '08, *Hofmeisters Beitr.*, 11, 415.

[16] Unpublished experiments.

These physical-chemical relations may offer an explanation of the extraordinary habit of certain blue-green algæ which normally thrive at a temperature of 68° C.[19] The water in which these algæ live contains numerous salts in solution and we suspect at once that among these salts there is a powerful liquefying agent which prevents coagulation by the abnormally high temperature, as in Pauli's experiments upon egg-white. We should expect that a reduction in temperature would prove fatal to such algæ since, under these altered circumstances, the liquefying agent would be free to act. So far as we know no experiments of this sort have ever been performed, though it may be significant that Setchell failed to find any algæ growing at 43°–45° C.

In a recent paper Osterhout[20] advances the hypothesis that substances which increase permeability antagonize those which decrease permeability. He says (p. 256):

> It seems to the writer that the hypothesis offers a rational explanation of antagonism by showing that salts antagonize each other because they produce opposite effects upon the protoplasm.

The nature of these "opposite effects upon the protoplasm" is an increase or decrease of permeability. Osterhout makes no statement as to the meaning of the term "permeability" which, without further qualification, is noncommittal, nor to the *cause* of the permeability changes. With these two fundamental gaps in the theory it seems a far cry to a "rational explanation of antagonism." We have emphasized above that a study of Osterhout's data indicates a direct correlation between decreased permeability and increased surface viscosity. It seems highly probable, however, that all substances producing an initial decrease in permeability will, if allowed to act long enough or in sufficient concentration eventually cause an *increase* in permeability.[21] This conclusion, which we are forced to make from a study of the phenomena of viscosity changes in colloids, complies very well with the experimental data upon permeability changes in both plant and animal cells.

All physical and chemical agents acting upon a colloidal system influence the state of aggregation of the disperse phase, tending either to increase or to decrease the degree of dispersion. Since we have a colloidal system at the surface of every cell, all physical and chemical agents influence the state of aggregation or its equivalent, the solubility of the surface disperse phases in one of two ways, viz., (1) there may be an increase in the degree of dispersion and a corresponding increase in the solubility of the disperse phases and the fluidity of the cell surface, or (2) there may be a decrease in the degree of dispersion or a decreased solubility of the disperse phases which eventually results in a precipitation or coagulation. An "antagonism" is to be considered a physiological compensation of a force favoring dispersion (solubility) by a second force favoring aggregation (insolubility). This relation is reciprocal.

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[17] It is impossible to carry out an experiment upon the melanophores of *Fundulus* which is directly comparable to Pauli's experiments on the elevated coagulation point of egg-white. KSCN produces a marked liquefaction upon the melanophores, but only after a relatively long exposure ($<$ 30 minutes). Atropine, on the other hand, brings about an irreversible disintegration at room temperature in concentrations of ca. 0.004 M in 0.1 M NaCl in about five minutes.

[18] See also Lepeschkin, W. W., '11, *Ber. d. deutsch. bot. Gesellsch.*, 29, 247; '13, *ibid.*, 30, 703.

[19] Setchell, W. A., '03, SCIENCE, 17, 943.

[20] Osterhout, *loc. cit.*

[21] Osterhout calls attention to this fact, but offers no explanation for it.

R. A. Spaeth

Osborn Zoological Laboratory,
Yale University

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110 (1174)

On the transformation of the plasma clot.

By **George A. Baitsell.** (By invitation.)

[*From the Osborn Zoölogical Laboratory, Yale University, New Haven, Connecticut.*]

It has previously[1] been shown by the author that in tissue cultures and in wound healing in the frog a fibrous tissue which is apparently identical with normal connective tissue may be formed by a direct transformation of a plasma clot. In an endeavor to analyze this reaction, plasma clots made from centrifuged blood plasma have been subjected to various conditions of tension and pressure. The results obtained show that with the aid of these mechanical factors it is possible to directly transform a typical fibrin net into a fibrous tissue. Judged from its histological structure when stained with Mallory's connective tissue stain, this new fibrous tissue is apparently identical with normal connective tissue of the frog. By varying the conditions it is possible to obtain preparations which will show various stages in the transformation ranging from a typical fibrin net to a fibrous tissue made up of bundles of wavy fibers such as is characteristic of normal connective tissue.

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[1] (*a*) *Jour. Exp. Med.*, Vol. 21, 1915, pp. 455–479; (*b*) *Jour. Exp. Med.*, Vol. 23, 1916, pp. 439–456.

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1915, xiii, pp. 189–193.

108 (1172)

Attenuation of the living agents of cyanolophia.

By **Rhoda Erdmann.** (By invitation.)

[*Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.*]

Paschen[1] (1911) says that leucocytes must play an important part in the process of immunization. This remark seems partly justified in the attenuation process of cyanolophia. The living agents of cyanolophia are differently affected in tissue cultures of *red* bone marrow from *white* bone marrow.

Experiment I, 1915.

Serum B Taken 36 Hours after Inoculation of Virulent Brain A in a Chicken, Shortly before its Death, Was Inoculated in a Tissue Culture of Red Bone Marrow and Chicken Plasma.

Length of Time in which Virulent Serum *B* was Cultivated in Tissue Culture at 30° C.	Record No. of Chicken.	Date of Death.	Length of Incubation.
Nov. 12–Nov. 15	No. 1	Nov. 18	48 hours
Nov. 12–Nov. 15	No. 3	Nov. 17	38 hours
Nov. 12–Nov. 18	No. 2	Nov. 21	72 hours

This proves that the virus can be kept alive six days at a temperature of 38° C. in a tissue culture of *red* bone marrow. Chickens No. 1 and No. 3 died in 48 and 38 hours. Chicken No. 2, which had been inoculated with serum B that had been kept 6 days in the tissue culture, died twenty-four hours later than the chicken which had been inoculated with serum B that had been only *three* days in a tissue culture of red bone marrow. This proves a certain attenuation by the cultivation of virulent serum in *red* bone marrow.

The living agents, which probably cause cyanolophia, can be cultivated in red bone marrow tissue cultures even longer than six days without losing their virulence.

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[1] Paschen, O., "Handbuch der Technik und Methodik der Immunitätsforschung," 1911.

Experiment XII, 1916.

Serum V Taken 36 Hours after Inoculation of Virulent Brain U in a Chicken, Shortly before its Death, Was Inoculated in a Tissue Culture of Red Bone Marrow and Chicken Plasma.

Length of Time in Which Virulent Serum *V* was Cultivated in Tissue Culture at 38° C.	Record No. of Chicken.	Date of Death.	Length of Incubation.
Feb. 23–March 2	No. 17	March 4	48 hours
Feb. 23–March 6	No. 18	Remained alive	
Feb. 23–March 11	No. 19	Remained alive	

So we can keep virulent the living agents of cyanolophia outside of the chicken 6–8 days at 38° C., yet in the tissue culture of *red* bone marrow the virus dies after 12 days. This is a perfect analogue to the experiment of Marchoux,[1] who cultivated the virus of cyanolophia in a culture medium which contained red blood corpuscles. He even believed that the living agents of cyanolophia had multiplied and produced a much stronger virus than that he inoculated in his cultures. In tissue cultures of red bone marrow no multiplication of the virus was observed, but a certain attenuation, as proved by the prolongation of the incubation period.

Different results were obtained by using *white* bone marrow.

Experiment II, 1915.

Serum C Taken 36 Hours after Inoculation of Virulent Brain B in a Chicken, Shortly before its Death, Was Inoculated in a Tissue Culture of White Bone Marrow and Chicken Plasma.

Length of Time in Which Virulent Serum *B* was Cultivated in Tissue Culture at 38° C.	Record No. of Chicken.	Date of Death.	Length of Incubation.
Nov. 30–Dec. 4	No. 3*a*	Living	
Nov. 30–Dec. 6	No. 4	Living	

Serum C, the Same as Used in Experiment Before, in Red Bone Marrow and Plasma.

Length of Time in which Virulent Serum *C* was Cultivated in Tissue Culture at 30°C.	Record No. of Chicken.	Date of Death.	Length of Incubation.
Nov. 30–Dec. 6	No. 5	Dec. 9	72 hours

This experiment proves that after 4 or 6 days in white bone marrow the virus is attenuated. After inoculation of virus in red bone marrow the animal died. A controlling experiment, in which serum C was used, which was kept 7 days on ice, showed that this serum killed the chicken after 38 hours, the usual time in which this strain of cyanolophia killed the animal. It was perfectly attenuated by the cultivation in white bone marrow, partially by cultivation in red bone marrow, and not at all by keeping the serum on ice.

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[1] Marchoux, "Cultures in vitro du virus de la peste aviaire," *Compt. rend. Acad. Sc.*, T. 147, p. 357, 1908.

The inoculation of attenuated serum and white bone marrow protected, to a certain degree, the chicken against a new inoculation, as the following experiments prove.

EXPERIMENT III, 1915–1916.

SERUM Y TAKEN 36 HOURS AFTER INOCULATION OF VIRULENT BRAIN H IN A CHICKEN, SHORTLY BEFORE ITS DEATH, WAS INOCULATED IN A TISSUE CULTURE OF WHITE BONE MARROW AND PLASMA.

Length of Time in which Virulent Serum *y* was Cultivated in Tissue Culture at 38° C.	Record No. of Chicken.	Date of Death.	Length of Incubation.
Dec. 26–Dec. 29	No. 3*a*	Living	
Dec. 26–Dec. 29	No. 4	Living	
Dec. 26–Dec. 29	No. 6	Died Jan. 1	72 hours

Chickens Nos. 3*a* and 4 had been inoculated with serum B and white bone marrow before, but not chicken No. 6. The same results were attained in experiments IV, V, VI, VIII, and X. All chickens which were treated with attenuated serum did not die when inoculated with a 2d or 3d dose of attenuated serum. All chickens which were not treated succumbed to the first doses of attenuated virus when it was kept only 2 or 3 days' time in tissue culture of white bone marrow. Always controlling experiments with the same untreated serum kept on ice were started, which killed the animals in due time.

It is possible to keep virulent the living agents of cyanolophia in plasma alone at a temperature of 38° for six days and longer, but the *same* virus dies in plasma, in which living white bone marrow is kept, in six days (experiment IX). The controlling experiment with serum, which was kept on ice, was positive, so it is true that the virulence of living agents of cyanolophia will be attenuated, and later die through the activity of the leucocytes. It was possible to shorten the length of time in which virulent serum was kept in tissue cultures of white bone marrow, and still inoculate it without success when the *treated animals were used again.* Animals 3*a*, 4, 7 and 8 survived after inoculation with serum M_1 which had been only 2 days in tissue culture (Experiment VI). Serum M_1 killed chicken M_2 after it was kept 5 days on ice, in due time. A shortening of the attenuation period to twenty-four hours was not sufficient to weaken the serum M_2. Chicken 7, which again was used, died in forty-eight hours, after having veen inoculated with serum M_2, that had only been one day in plasma and white bone marrow.

Steinhardt and Lambert[1] cultivated the living agents of vaccinia in tissue cultures of rabbit cornea. They report a definite increase of the virus, as measured by the effects of successful reinoculations. Growth of the virus could be observed in tissue cultures of the rabbit's cornea only, while heart, kidney and liver gave no results. My experiments, previously reported, prove a rapid attenuation of the virus in *white* bone marrow tissue culture. This is quite remarkable, because the living agent of cyanolophia is not surpassed in virulence by any other virus.

The next series of experiments will deal with the attenuation of the living agents of cyanolophia in brain and liver tissue cultures and with the importance of these and the white bone marrow tissue cultures for active immunization.

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[1] Steinhardt, E., and Lambert, R. A., "Studies on the Cultivation of the Virus of Vaccinia, II," *Journ. of Inf. Diseases*, 1914, Vol. 14, pp. 87–92.

Reprinted from the Proceedings of the Society for Experimental Biology and Medicine, 1916, xiii, pp. 181-182.

103 (1167)

Conduction, excitability and rhythm-forming power of the atrio-ventricular connection in the turtle.

By HENRY LAURENS.

[*From the Osborn Zoölogical Laboratory, Yale University.*]

As in the heart of the turtle *Clemmys lutaria* and of the lizards *Lacerta viridis* and *agilis* (Laurens[1]) the right and left parts of the atrio-ventricular funnel of *Malacoclemmys geographica* are the portions which are most efficient in conducting the contracting impulse from the auricles to the ventricle. When the auricles are partially separated from the ventricle by a series of cuts leaving only a narrow connection, and in consequence of which atrio-ventricular block (complete or incomplete) has been brought about, it is these parts which are later most capable of conducting the impulse from the auricles to the ventricle so that the contractions of the latter follow those of the auricle coördinatedly, or so that the incomplete block is decreased.

Stimulating the funnel of beating (in situ and excised) and still hearts (first Stannius ligature) with single shocks (quick make and break) and with interrupted currents of short duration have shown (1) that the funnel is more easily excited than the base of the ventricle, (2) that the right and left parts of the funnel are more easily excited than other parts (dorsal and ventral) and (3) that the excitability of the funnel increases as one approaches the auricle.

The stimulation of the funnel just below the level of the A-V boundary of beating hearts with interrupted currents, even when these are strong and of long duration, can only occasionally produce a "fibrillation" of the ventricle or a V-A rhythm (funnel rhythm) which lasts over after the stimulation is discontinued. This is possible, however, and curves have been obtained from excised hearts showing a duration for several seconds of a funnel rhythm following a ventricular "fibrillation." In the still heart the setting up of a funnel rhythm is more easily and frequently accomplished, and several cases have been registered showing a funnel rhythm lasting for several minutes.

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[1] Laurens, *Pfluger's Archiv*, 1913, 150, p. 139.

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1916, xiii, pp. 182–183.

104 (1168)

The influence of the vagi and of the sympathetic nerves on the rhythm-forming power of the atrioventricular connection in the turtle.

By **Henry Laurens** and **C. C. Gault.**

[*From the Osborn Zoölogical Laboratory, Yale University.*]

The investigations here reported were undertaken to determine the action of the vagus and sympathetic nerves upon the V-A rhythm produced by electrical stimulation of the atrioventricular funnel. In *Malacoclemmys geographica* the two nerves are not fused into a single trunk, but run separately in the neck just median to the carotid artery. The turtles were decerebrated, and the plastron removed, the circulation being kept intact to a large degree. The vagus was stimulated just above the thoracico-abdominal ganglion, and the sympathetic, between the median cervical and the first thoracic ganglion.

Stimulation of the vagus nerves alone gave the usual results. The effects of sympathetic stimulation were, however, not so clearly marked. The general effect was a slight augmentation of the auricular contractions. Acceleration of the heart beat was less frequently obtained, the average being from 2 to 3 beats per minute, although an acceleration of as many as 6 beats per minute was registered.

Conjoint stimulation of the vagus and the atrio-ventricular funnel just below the A-V boundary with relatively strong interrupted currents produces a V-A rhythm which lasts over, in different experiments for varying lengths of time, after the stimulation has been discontinued. In these cases stimulation of the vagus nerves with a current of sufficient strength to still the normal heart causes only a decrease in the height of the auricular contraction with no effect on the rate of beat. Stimulation of the sympathetic with strong currents stops the funnel rhythm, after which a normal atrio-ventricular beat begins.

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105 (1169)

Changes in form and position of the retinal elements of normal and transplanted eyes of Amblystoma larvæ occasioned by light and darkness.

By Henry Laurens and J. W. Williams.

[*From the Osborn Zoölogical Laboratory, Yale University.*]

In order to investigate the changes occasioned by light and darkness in the retinal elements of a Urodele a series of experiments on large (37 to 45 mm.) larval and on recently metamorphosed individuals of *Amblystoma* was carried out. It was found that the pigment of these eyes undergoes a decided forward movement when the animals are transferred from darkness to light. In darkness most of the pigment is massed near the base of the epithelial cells, and only comparatively few needles extend into the protoplasmic processes between the visual cells. In light a decidedly greater amount of pigment moves toward the external limiting membrane so that the basal layer is thinner. Measurements of the distance from the external limiting membrane to the nearest pigment needle (or from the choroid edge of the epithelial cells to the farthest pigment needle) are practically the same in light and dark eyes, so that this kind of measurement gives no indication of the extent of movement of the pigment.

The cones in the light eye are 4.2 μ shorter than those in the dark eye, the total expanded length of the ^ cones being 25 μ. The rods seem to be longer in the light eyes than in the dark, but the increase is too slight to permit of satisfactory measurement.

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Optic cups were transplanted at the tail bud stage to various parts of the body, where they developed to form more or less perfect eyes. The region of the auditory vesicle seemed to offer a particularly advantageous spot for the transplant. In the transplanted eyes the movement of the pigment is fully as great as in the normal eyes. The cones also contract in the light but only to the extent of about 2.5 μ.

Pigment migration and cone contraction therefore do take place in a Urodele retina and can do so independently of the central nervous system.

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CONTRIBUTIONS TO THE STUDY OF CELL MECHANICS

I. SPIRAL ASTERS

THEOPHILUS S. PAINTER
(Instructor in the Sheffield Scientific School)
From the Osborn Zoological Laboratory, Yale University

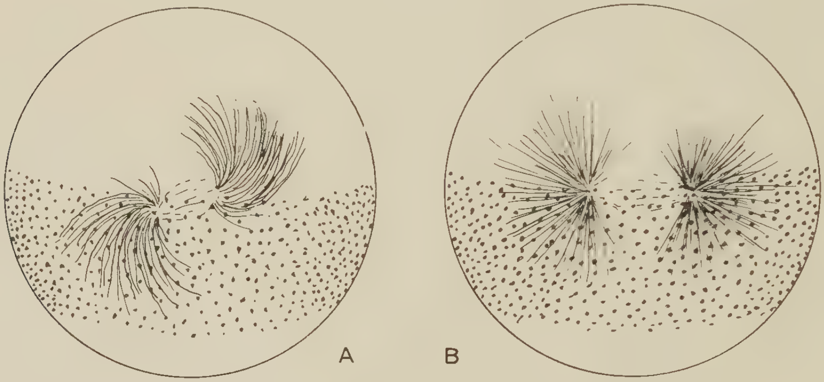
SEVEN TEXT FIGURES AND TWO PLATES

While making an experimental study of sea urchin eggs in Naples, during the spring of 1914, it was the good fortune of the author to observe one case of a 'spiral aster.' The egg in which this was seen had previously been treated with a dilute solution of phenyl urethane shortly after fertilization, and had subsequently been washed free of the narcotic.

Since taking up a cytological study of the sea urchin material, collected at Naples, a number of asters with bent or twisted rays have been observed to occur at a definite period in the development of certain monaster eggs. That these stages are not due to faulty preservation is proved from several considerations. First, the phenomenon has been observed and followed in the living egg. Second, it appears, as we shall see, at definite phases in the development of certain monaster eggs in two separate series of material preserved at different times. Third, one may trace the history of the asters from the time when the first bending appears until it finally disappears.

The facts, in the case of the sea urchin, pointed so clearly to one interpretation only, that I took up a review of the more important papers where spiral asters have been recorded in order to see in how far the explanation arrived at from the study of Strongylocentrotus would be applicable to the observations of other workers dealing with widely different forms. The result has been so satisfactory that it seems worth while to describe in detail the phenomena observed in the eggs of Strongylocentrotus lividus and to show how the interpretation of the facts here throws light on spiral asters; a phase in the history of the centrosome which has been a dark chapter since Mark, in 1881, first described and figures 'spiral asters' for Limax campestris.

The monaster eggs, in which the phenomenon has been observed, were produced by shaking violently a few minutes after the fertilization membrane had been formed. Series were preserved at frequent intervals (from 3 to 15 minutes depending on the stage) either in Boveri's picro-acetic mixture or in sublimate acetic. Sections were cut at 7 μ. The stains used were Heidenhain's haematoxylin followed by Lichtgrün.



As previously mentioned the living egg which showed the spiral aster had been treated with phenyl urethane and then washed in pure sea water. When first observed, the majority of the eggs (treated in the same way) were in the 2- and 4-cell stages. This one egg, however, was undivided and judging from the excentric position of the spindle, had passed partially through a monaster cycle. (The treatment of the eggs with this narcotic frequently produces monasters.) Two general centers of radiations were seen, with a very faint spindle between them. The rays were very much bent, as may be seen from figure A, and the spindle itself lay at an angle to the pigment band.[1] As the egg was followed under high power, the two asters shifted their positions until the spindle lay in the plane shown in figure B. The bending of the rays soon disappeared and the egg divided.

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[1] The eggs of the female upon which this experiment was made showed the pigment ring very clearly. This allowed me to mark the position of the spindle.

A short description of the behavior of monaster eggs, produced by shaking, has been given by Boveri ('03), and certain phases of their history has been taken up by the author ('15). From these descriptions, it is clear that while most of the monaster eggs pass through a cycle of changes comparable to the changes going on during normal cleavage, a few shift directly into an amphiaster, a short time after the monaster is formed, and divide. It is from the eggs of this class that spiral asters are derived.

The first sign of the formation of a spiral aster is found in typical monaster eggs at the period when the controls are in the early 2-cell stage. An egg is shown in figure 1 in which a slight bending of the rays may be seen (to the left in the figure). A careful inspection of the figure will show that these few bending rays arise from the edge of the centrosphere, run a trifle forward for perhaps a third of their course, and then bend backwards. In other respects, the egg is a typical monaster with the chromosomes in this case divided forming a half-hemisphere around the aster.

In figure 2 an egg is shown where the bending process has become very pronounced. Here we note that the centers of twisting are localized at two opposite points on the centrosphere. In these regions, the rays arise from the edge of the sphere, run forward for a half of their length, perhaps, and then backwards, that is, opposite to the direction in which the aster seems to be moving. It will be seen, also, that the two centers are bending in the same direction, in this case to the right (clockwise). Those portions of the aster which are not involved in the active movement show the rays bending uniformly backwards. Judging from the position of the chromosomes, the egg is a monaster which has been cut equatorially.[2] The whole appearance of the figure is what might be expected were there two areas of movement just outside of the centrosphere pushing the rays forward in these regions and dragging the whole aster along with it.

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[2] This does not refer to the polarity of the egg. In monaster eggs the chromosomes lie on one side of the aster. The plane of section has cut the egg in such a way that we see the aster in the center and the chromosomes on both sides of it.

Figure 3 shows an egg in which the bending of the rays is still more pronounced. The two areas of twisting are to be made out, less clearly than in figure 2, and a careful inspection shows that the rays in these two regions run forward and then backwards as described for the other cases. This egg is particularly interesting as it shows how the astral rays have been pulled free from their anchorings in the cytoplasm. This egg shows, perhaps, the condition most commonly found.

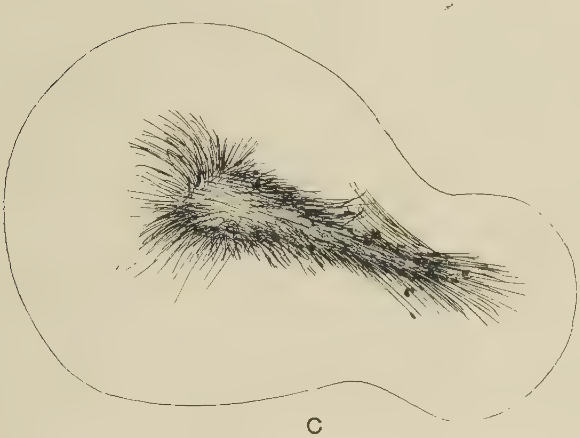
In figure 4 we see an egg in which the twisting is very clearly localized around two points. The section cuts the aster somewhat obliquely so that only one center shows distinctly. A faint spindle may be seen in the centrosphere connecting the two centers. Again we note that the course of the rays at the two centers of movement is the same as in the other eggs. One further point of interest lies in the position of the chromosomes. It will be noted that in the region of bending two chromosomes have been pulled away from their mates.

Figure 5 shows an egg in which the two centers have moved far apart. Faint traces of the centrosphere may still be seen connecting them. The chromosomes lie, for the most part, around one center. (This is shown both in this section and in those adjoining it.)

Figures 6 and 7 show later stages of the spiral asters. In figure 6 the chromosomes are scattered, though they lie in the plane of the spindle connecting the two centers. In figure 7, an equatorial plate has been formed. (In the latter case the plane of section is a trifle oblique to that of the spindle.)

In figure 8 we have an egg which appears to be the final stage of the spiral aster. The rays are no longer bent, they appear somewhat finer than those of the preceding figures. The only trace of the bending is seen in the spindle itself. A division of the chromosomes has taken place, though the latter fact is not clear from the figure.

The eggs shown in the figures, with the exception of 3, perhaps, seem to form continuous steps in a process of shifting of the position of the spindle in the egg. Judging from the large number of cases observed similar to these figures, the usual behavior of spiral aster eggs is that shown. Here and there, however, eggs, which have apparently arisen from those in which the rays were bent, are found which have a different history. Typical cases are shown in figures C, D, and E. The eggs shown in figures C and D, may be derived, perhaps, from eggs similar to the one shown in figure 5, in which the chromosomes are preponderantly on one side. In this event, a true spindle is not formed, but the one center goes apart from the other dragging with it a few chromosomes, as shown in figure C, and an abortive attempt at division is made. Figure D shows a stage of this. Figure E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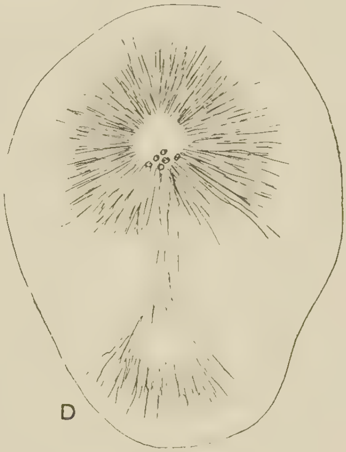
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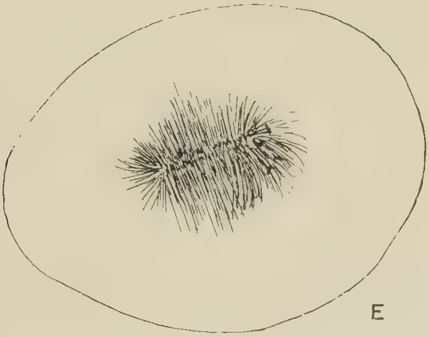

E

shows a type of egg, perhaps some phase in the development of those shown in the foregoing text figures, which is often seen. The centrosphere has collapsed or become greatly flattened and the bending of the rays is seen at one end. What the ultimate fate of these eggs is I am unable to say. At the time the living material was being observed, I was not aware of the occurrence of eggs of this type. Cases were noted here and there in the living material in which the protoplasmic streaming was very severe and it is possible that such eggs had the history shown in figures C and D.

The fate of the spiral aster eggs shown in figures 1, 2, 4, 6, 7, and 8 seems to be a more or less normal cleavage. This conclusion rests on the fact that in the living material those eggs which shifted early from a monaster into an amphiaster divided normally. In one case only have I actually followed the division with high power. That was in the egg treated with phenyl urethane. It might be asked why these monaster eggs dividing early were not followed more closely? It must be admitted that at the time the experiments were being made, the points of greatest interest were centered in those eggs which passed through a complete monaster cycle. In the average experiment, one obtains from 5 to 10 per cent of the egg showing the single aster and of these, perhaps one in twenty shifts early into an amphiaster and divides. These cases were thus so rare that no attempt was made to follow them under higher powers, in order to see the condition of the astral rays.

The stages with the twisted rays are found for a very short time in both series studied. They appear when the controls are shifting from the 2- to the 4-cell stage. No evidence of eggs with bent rays has been found at any other period. That the eggs which show spiral asters come from monaster eggs which shift over into an amphiaster is clear from the following considerations.

When the monaster eggs are first seen in section the centrosphere is round. At a later period one finds the sphere elongated and a careful study of such eggs has shown that a division of the centrioles has taken place. A stage of this is shown in figure 9.

This egg is typical of a number of monasters examined at this time. We see the two centrioles connected by a spindle with new rays arising from the new centers. The chromosomes remain attached to the old fibers which are still very prominent. The centrosphere elongates still more and the new centers can no longer be made out. Up to this time no spiral asters are found in the sectioned material. Following this stage, however, we find the eggs figured in the plates, with the exception, of course, of figure 9.

The figures of the eggs themselves show further that two centers are involved in the spiral aster eggs. Since these stages follow on the division of the centrioles, it can scarcely be doubted that, in the former eggs, the centrioles lie at the two points of the centrosphere where the areas of twisting are greatest, as in figures 2, 3, 4, and 5.

We now have the facts necessary in order to understand what is taking place in the eggs which show spiral asters. In the living egg, owing to the happy circumstance that the pigment ring was prominent, it was possible to see that a shifting of the position of the asters and of the spindle was taking place. From a plane which formed a considerable angle with the pigment band, the spindle shifted until it lay parallel to it. In the preserved material, we find every phase of this shifting; and the majority of the eggs show clearly that two centers are involved, from which we may safely conclude that the division of the centrioles normally precedes the shifting process. There are several phases of the shifting which warrant consideration.

In the foreground stands the question, How is this shifting of the spindle brought about? Do the new centers, the centrosomes, move first, or, are they carried along by the protoplasm lying outside of the centrosphere? The answer to this question is given by a close study of the astral rays. In all of the figures the point has been emphasized that, in the region of the greatest bending, the rays run forward for a part of their length and then backwards. It is clear from the considerations given above that these areas of twisting are associated with the new division centers. Now, were these centers themselves the first points to move, we should expect that the rays would bend more or less uniformly backwards from these points, certainly, under no circumstances would they bend forward and then backwards. The latter conditions could only come about when some region outside of the centrosphere was the first to move. And, if we may judge from the rays in the spiral aster eggs, the areas of movement are here situated about a third of the ray's length from the centrosphere. It is only by assuming this that we can understand the reason that the rays run forward and then backwards. With two areas in the protoplasm moving, the rays would be pushed forward in these regions and the centrosphere would be dragged along, carried simply by the protoplasm. In the regions where the protoplasm was not so actively moving the rays would bend uniformly backwards. That this is the true state of affairs, a careful examination of the figures will show.

What part the new division centers play in this shifting process, we are unable to say. That the movement is coincident with the division of the centrioles is clear, but whether the one is the cause and the other the effect, or whether they are both the effect of some common cause, is a question upon which the present work throws no light.

A second point of interest lies in the relation between the new and the old rays, for, with the division of the centriole in the monaster we have the formation of new rays, as figure 9 will show. It is in this respect that the conditions in the monaster and in the normal eggs are different. In the normal eggs at the time when the centrioles divide and go apart, the radiations from the old aster have become very faint or have disappeared. In the monaster egg, on the contrary, the centrioles may divide at the time when the radiations from the aster have reached their maximum intensity. It is for this reason, I think, that we find spiral asters in these eggs. Following the division of the centrosome new rays appear. They are concealed from view by the highly developed rays of the old aster. When the new centers shift their positions, the old rays tend to retard the movement and thus we get the peculiar figures decribed above.

A further question of interest is, what significance has the shifting of the spindle in the monaster eggs? The answer is given by a consideration of several facts. Boveri ('05) was the first to suggest that the cytoplasm in the sea urchin egg undergoes a series of changes—a sort of development—independent of the nuclear elements, and I reached the conclusion, after studying eggs treated with narcotics that "at the time of fertilization progressive changes, which go on independently of the nucleus and of cleavage, are initiated in the cytoplasm of the eggs and that these changes determine the position of the spindles in the egg and consequently in its blastomeres," p. 299 (l. c.).

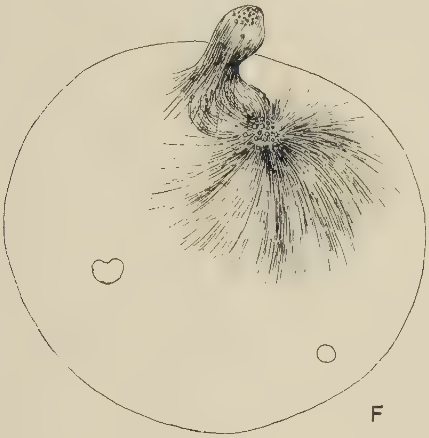
In monaster eggs the division mechanism is delayed through the failure of the centrosome to divide, but the cytoplasmic development is not materially interfered with. This is indicated by the fact that if the aster recovers and divides into an amphiaster, the spindle tends to take up the same relative position as the normal controls have taken and as a consequence we have the early production of the micromeres as Boveri ('05), and I myself have shown.

The single aster, during its early history takes up a position at or near the center of the egg. The egg cytoplasm, after fertilization, begins its development which normally, of course, goes hand in hand with certain changes in the nucleus and of the division mechanism. By the production of monasters we are able to separate, for a time, the division mechanism and the cytoplasmic development. When now the division mechanism is released, as through the division of the centriole or the completion of the monaster cycle, the new spindle tends to take up the same position which the control eggs have. Our observations show that the spiral asters arise from monaster eggs which have recovered very early from the effects of the shaking, when the controls are in the 2- and 4-cell stages. From this we should expect that the young spindle will attempt to take up the position which the controls possess, that is, either in the plane of the first or of the second cleavage. This expectation is fulfilled by the single living egg which was observed. The young spindle, resulting from the division of a monaster, lay at an angle with the pigment band, at a time when the controls were in and 2- and 4-cell stages. Boveri ('01) showed that the first two divisions in Strongylocentrotus were meridional, that is, cut the pigment band at right angles. In the living egg the spindle did shift until it occupied the position in which the pigment band would be cut, either in the first or second cleavage plane. That the eggs found in a study of the sections show phases of this shifting process is scarcely to be doubted in the light of all the facts.

A number of authors have recorded the appearance of spiral asters in the eggs of widely separate groups; Nemerteans, Molluscs, Annelids, and in at least one vertebrate, Axolotl (Fick, '93). The phenomenon has been more frequently described for the molluscs, and it seems confined to the gastropods. Mark ('81) was the first to describe and figure spiral asters, in Limax campestris. In this form they appeared, somewhat inconstantly, at the time of the second polar body formation. At least four other authors have recorded spiral asters in various snails at this period of the maturation. Kostanecki and Wierzejski ('96) mention it for Physa. MacFarland ('96) observed and has given excellent figures of it in Pleurophyllidia; Byrnes ('99) decribes it for Limax agrestis; and Linville ('00) notes its occurrence in Limax maximus.

None of these authors have attempted to explain the cause of the spiral aster. Conklin ('02) and ('05), however, has described in detail the movements taking place in the protoplasm of the eggs of Crepidula, and the Ascidian cynthia during maturation and cleavage. From the observations of this author it is clear that materials are constantly being shifted from one part of the egg to another in both of these widely separated forms. Kostanecki and Wierzejski have noted that the same was true of Physa. Conklin points out, in discussing these movements "that the movements within the cell substance of the unsegmented egg are, in certain cases at least, of a vortical character is indicated by the spiral asters, first described by Mark for Limax, and since observed by several other investigators in other animals, and also by my observation that the first cleavage in Crepidula is a spiral one, being oblique to the right or dexiotropic" (p. 79, Karyokinesis and Cytokinesis).

That the peculiar bent fibers are not caused by the movement of the division center itself, in the case of the gastropods, is shown by the figures of both Mark and MacFarland. I reproduce figure 66 from Mark's paper in figure F. Here it will be noted, first that the egg aster does not lie in a normal plane, but has been shifted to one side. And secondly, that the rays themselves run forward and then backwards. The figures of MacFarland show the same thing for the rays in the eggs of Pleurophyllidia. I have explained how such a course of the fibers, in the case of the sea urchin, is incompatible with the view that the asters themselves move first. It is clear that, as Conklin has shown, the movements of the protoplasm are responsible for the bending of the rays. The reason that the twisting of the rays has been described so often for gastropods doubtless lies in the fact, that as Linville has pointed out, the egg aster persists for an unusually long time after the second polar body is given off. Byrnes has given figures which show very plainly the persistence of the rays until the pronuclei are uniting.



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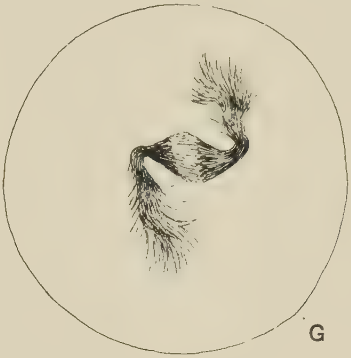

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Coe ('99) in working on the maturation and fertilization of Cerebratulus observed many cases where the fibers running from the centrosphere were bent. The most interesting case, perhaps is that shown by the author in figure 37, plate 21, which I have copied in figure G. A glance at this figure will show that the two division centers are being carried away from the positions which they occupied when the spindle was first formed. In the light of the facts recorded above, the explanation of this condition seems clear. We are dealing here, I believe, with a case of delayed cleavage. The first two cleavage planes, in Cerebratulus stand at right angles to each other, just as in the case of the sea urchin. This egg was delayed for some reason in its division, after the spindle was formed, and we see the asters being carried by protoplasmic currents to the new plane where the division of the protoplasm should occur.

Among certain of the leeches, the spiral aster seems to be a normal feature in the development of definite blastomeres and in the polar body formation. Iijima ('81) figures it in eggs before the polar bodies have been given off, but his description is too meagre to allow us to draw any conclusions as to the cause. Later than this, we have the works of Sukatschoff ('03) on the development of Nephelis. This author figures spiral asters as a constant feature in the development of certain blastomeres and advances, tentatively, an explanation for this. After pointing out that we frequently have a shifting of blastomeres during development he suggests that the two cells where he has constantly found the spiral asters rotate on each other. "Nehmen wir nun ferner an,—was a priori nicht ausgeschlossen erscheint—, dass die äusserste plasmatische Schicht der Zelle in sich eine grössere Kohäsion besitzt, als das innere Plasma, so müssten natürlich die Vorher gerade verlaufenden strahlen in der Richtung der Drehung spiralig gedreht werden (p. 333, l.c.).

This author admits, however, that his explanation will not apply to those cases where the spiral asters appear during polar body formation.

A glance at the figures given by Sukatschoff shows that the rays of the asters run in the same way that I have described for the sea urchin, that is, forward for a part of their length and then backwards. It is scarcely to be doubted that the cause of the bent rays lies in the protoplasmic movement of certain parts of the cell outside of the asters themselves. Since the turning is a constant feature for certain blastomeres, it probably has some significance and is not due simply to a rearrangement of materials, as Conklin found, for example, in Crepidula. It may be possible that it is a reminiscence of a former cleavage which has now been omitted from the ontogeny of Nephelis. This explanation does not seem improbable. However this may be, the main point for us is that since the rays run forward and then backwards, the cause of the bending can not lie in the asters themselves but must be in the protoplasm outside of these division areas.

This short review of a few of the papers dealing with spiral asters (I have made no effort to give a complete bibliography) is sufficient to show that the conclusion reached, after a study of the sea urchin egg, is valid for a number of other forms.

There are several points of general interest which should be mentioned here, as our observations throw some light upon them.[3]

That spindles shift during the development of eggs, especially during polar body formation, has been observed by a great number of workers. It has been generally accepted, that this shifting was brought about by the protoplasm and not by the asters themselves, but this has been founded on indirect evidence only. The observations of the sea urchin egg allow us, I think, to understand the time and the nature of the movement a little more clearly. The conditions in eggs, in which there is a rotation of the spindle of the second polar body, and eggs which shift from a monaster to an amphiaster are similar in this respect, that we have spindles which must shift to take up new positions. Ordinarily, this shifting takes place without any bending of the rays of the young asters in the egg and we have no indication of the cause of the movement. But in monaster eggs, the high development of the old rays allows us to see where the movement begins. An examination of the figures given will show that the areas of movement lie well outside of the centrosphere in the cytoplasm. Were not old fibers present, there would be nothing to indicate that a shifting of the cytoplasm was taking place, and the spindle would probably rotate until the proper plane was reached and then the egg would divide.

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[3] I have not touched upon the nature of the fibers in the sea urchin egg, in this paper, though the spiral asters throw much light upon this question. In a work shortly to be published, a complete analysis of monaster eggs will be given. I propose to take up in this the nature of the rays.

In its broader aspect, the present contribution adds just one more bit of evidence to show how very important is the rôle which the cytoplasm plays in development and how very complex it is in its organization. The sea urchin egg, was at first considered as one of the simplest types; it seemed certain that, if it was possible anywhere to reduce certain phases of development to simple mechanical terms, it could be done here. But a mass of experimental data has gone to prove that, in many respects, this apparently simple egg is fully as highly organized as seemingly complex eggs such as the ascidian, for example. The cytoplasm can no longer be looked upon as so much passive material which the nucleus elaborates during development, but it has a complex organization and a development which may be synchronous with certain changes in the nucleus and the division mechanism, and yet is independent of these.

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PLATE 1

EXPLANATION OF FIGURES

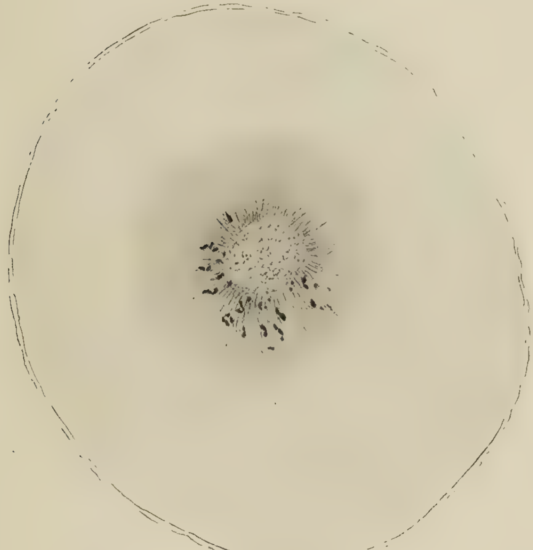
All drawings were made under oil immersions with the aid of a camera lucida, by the laboratory artist, Miss Krause.

1. Monaster egg showing the first signs of the bending of the rays.

2. A monaster egg showing that there are two centers involved in the twisting process.

3. A later stage of the same

4. An egg to show the two areas of movement and the faint spindle in the centrosphere.



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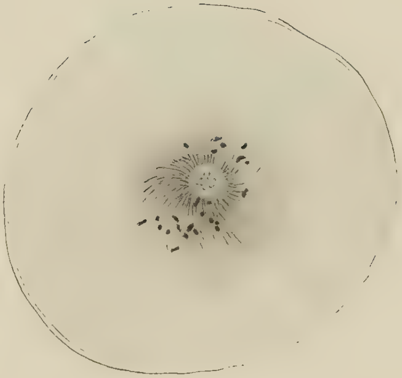

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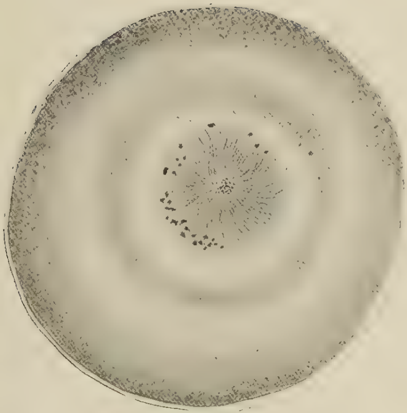

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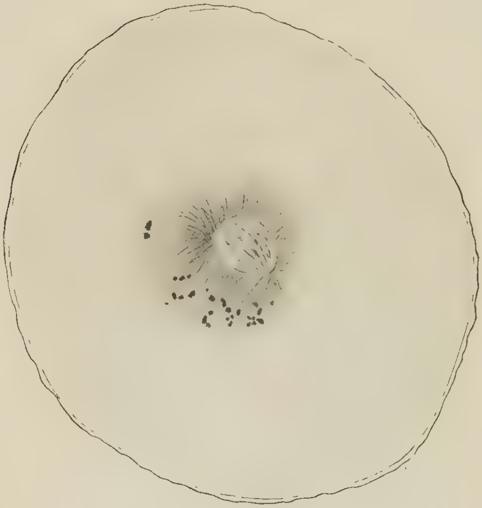

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PLATE 2

EXPLANATION OF FIGURES

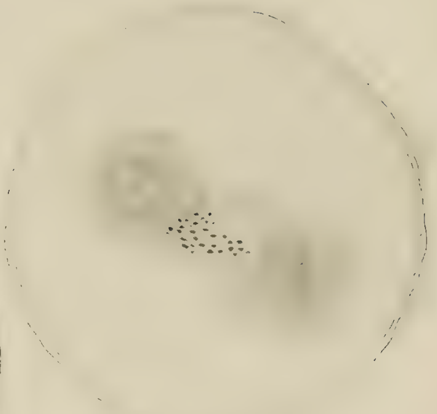
5. A later stage showing the two points of movement well separated.

6, 7, and 8 show three successive stages in the formation of a new spindle.

9. Showing the division of the centrioles in the monaster egg.

Fig. F Showing second polar body for motion in Limax campestris. After Mark.

Fig. G A first cleavage spindle in Cerebratulus. After Coe.



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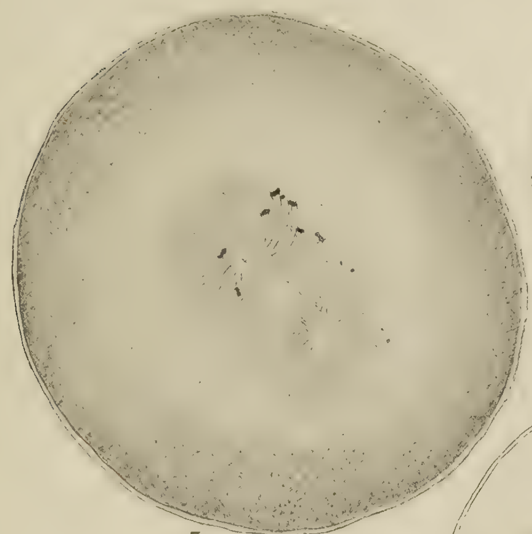

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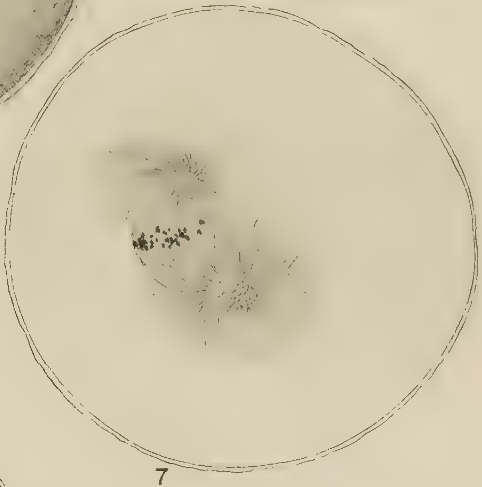

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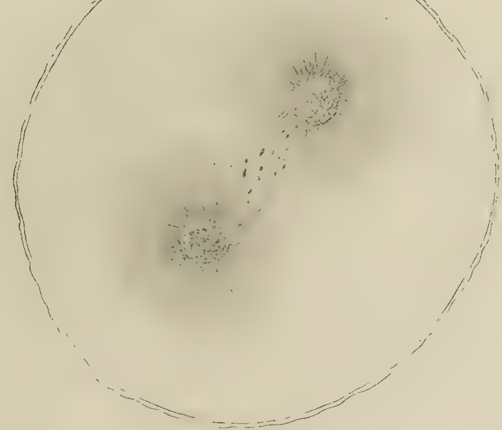

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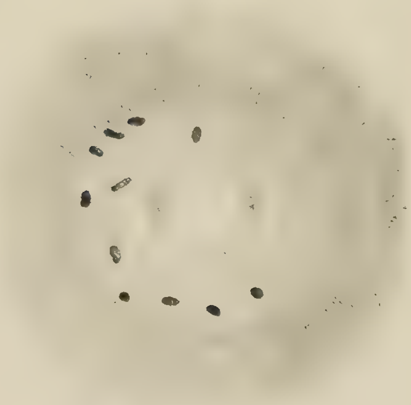

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Reprinted from the Proceedings of the Society for Experimental Biology and Medicine, 1916, xiii, p. 193.

109 (1173)

Experiments on the physiology of digestion in Blattidæ.

By **Eldon W. Sanford.** (By invitation.)

[*From Osborn Zoölogical Laboratory, Yale University.*]

The question as to whether fat is digested and absorbed in the crop of the cockroach was answered in the affirmative by Professor Petrunkevitch in 1898, but in the negative by more recent authors. My investigations, which were done under the direction of Professor Petrunkevitch, show that fat is split to soluble products and absorbed in large amount in the crop, the process being observable as gradually more and more in the crop's epithelial cells at successive intervals up to forty-eight hours, and gradually less afterward. Some cells absorb so much that they appear solid black when stained with osmic acid. Ligation of the crop from the stomach does not hinder or modify the process. Fatty acids are absorbed like fats.

After certain intervals after fat feeding much fat is found in the tracheal tubes, sometimes filling them, sometimes in a thin layer on their walls, sometimes only on the supporting spirals, and sometimes mingled with chyme. This chyme resembles that normally present in the crop lumen; it is regularly present in some of the tracheaæ, and in it leucocytes are often found. The chyme is evidently a normal content. The fat enters the tubes through the tracheal end cells, after being absorbed by them from the lumen of the crop.

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113 (1177)

The response of single cells to electrical stimulation.

By R. A. Spaeth. (By invitation.)

[*From the Osborn Zoölogical Laboratory, Yale University, New Haven.*]

There is an accumulation of embryological, morphological and physiological evidence at hand showing that the melanophores of vertebrates are to be considered highly modified smooth muscle cells. By means of a simple recording device the responses of single melanophores of *Fundulus heteroclitus* to faradic and galvanic stimulation have been studied in some detail.

In faradic stimulation it appears that as regards the duration of the latent period, the quantity of current necessary to bring about a response in the cell, the increased height of the contraction curve with an increase in the strength of stimulus and the development of tetanus by properly spaced single break shocks, the contraction curves for a single melanophore show a striking resemblance to smooth muscle graphs obtained from the bladder of the cat (Stewart) and the stomach of the frog (Howell).

A constant current, which has previously been supposed to produce an expansion of the melanophores, causes a contraction when applied through non-polarizable electrodes of the $Zn\text{-}ZnSO_4$ type. An expansion of the melanophores may be produced by galvanic stimulation if platinum electrodes are used but this has been shown to be due to hydroxyl ions liberated at the cathode. Both the make and the flow of the constant current are effective contracting stimuli. With currents of moderate strength there is, at first, a rapid rise in the contraction curve due to the combined effects of make and flow but subsequently a partial falling off of the contraction giving a typical plateau. Stewart has found precisely the same conditions in the bladder of the cat. No response to the breaking of the constant current has thus far been observed in the melanophore.

The evidence obtained from these experiments with the responses of single melanophores to electrical stimulation, appears to strengthen and corroborate the writer's contention that in the melanophore we are dealing with a modified and disguised type of smooth muscle cell.

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89 (1153)

Endomixis in diverse races of Paramaecium aurelia.

By **Lorande Loss Woodruff.**

[*From the Osborn Zoölogical Laboratory, Yale University.*]

Woodruff and Erdmann in 1914[1] described a normal periodic reorganization process without cell fusion, which they termed endomixis, in *Paramaecium aurelia*. This study was based chiefly on pedigreed cells from Woodruff's 5,000-generation race of *Paramaecium aurelia*, though specimens of a race of this organism isolated by Erdmann in Germany showed the same phenomenon.

The present communication is to prove the general occurrence of endomixis in races of *Paramaecium aurelia*, since this has been questioned, on a priori grounds, by certain authors.

The following races of *Paramaecium aurelia*, in addition to those mentioned above, have now been studied:

Oberlin Race. Isolated at Oberlin, Ohio. Carried in pedigreed culture from October 8, 1914, to date, during which time it has attained 951 generations.

Bryn Mawr Race. Isolated at Bryn Mawr, Pa. In pedigreed culture from January 7, 1915, to February 8, 1916, when it was discontinued at the 650th generation.

Oxford Race. Isolated at Oxford, Ohio. Pedigreed culture started on July 16, 1915, and has to-day (May 24, 1916) attained the 779th generation.

Woods Hole Race. Isolated at Woods Hole, Mass. Pedigreed culture begun on August 11, 1915, and discontinued on January 14, 1916, at the 305th generation.

Each of the above races has shown endomixis at the regular rhythmic periods throughout its culture and therefore this additional data from races from diverse sources fully corroborates the statement of Woodruff and Erdmann[1] that "this reorganization process is a normal phenomenon and probably occurs in all races of the species *Paramaecium aurelia.*"

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[1] *Loc. cit.*

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[1] Woodruff and Erdmann, "Complete Periodic Nuclear Reorganization without Cell Fusion in a Pedigreed Race of *Paramaecium,*" PROC. SOC. FOR EXPER. BIOLOGY AND MED., Vol. 11, 1914 (preliminary paper). Erdmann and Woodruff, "Vollstandige periodische Erneuerung des Kernapparates ohne Zellverschmelzung bei reinlinigen Paramaecien," *Biol. Centr.*, Bd. 34, 1914 (preliminary paper). Woodruff and Erdmann, "A Normal Periodic Reorganization Process without Cell Fusion in *Paramaecium,*" *Journal of Exper. Zoology*, Vol. 17, No. 4, 1914 (complete paper).

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THE ORIGIN AND STRUCTURE OF A FIBROUS TISSUE FORMED IN WOUND HEALING.

BY GEORGE A. BAITSELL, PH.D.

(*From the Osborn Zoological Laboratory of Yale University, New Haven.*)

PLATES 106 TO 109.

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INTRODUCTION.

In a previous paper[1] the author showed that in living cultures of adult frog tissues there occurs, in many instances, a transformation of the plasma clot in which the living tissue is imbedded. This transformation results in a consolidation or fusion of the elements of the fibrin net and a consequent formation from it of a fibrous tissue which is identical in its form and structure and in many of its staining reactions with a regular collagenous connective tissue. It was felt that, whether or not this new fibrous tissue directly formed from the fibrin clot remained as a permanent connective tissue, such a reaction must play a fundamental part in the processes attendant upon wound healing. The present paper[2] gives the results obtained from an extensive series of experiments undertaken for the purpose of studying the action and fate of the fibrin clot formed during wound healing, and they give evidence that in the healing of skin wounds in the frog a definite transformation of the fibrin clot takes place such as was found to occur in the tissue cultures. This transformation also results in the formation of a new fibrous tissue, without intracellular action, which is apparently identical with regular permanent connective tissue.

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[1] Baitsell, G. A., The Origin and Structure of a Fibrous Tissue Which Appears in Living Cultures of Adult Frog Tissues, *Jour. Exper. Med.*, 1915, xxi, 455.

[2] A report of this work was given at the meeting of the American Association of Anatomists held at New Haven, Conn., Dec. 28–30, 1915. An abstract appears in the proceedings in the *Anat. Rec.*, 1916, x, 175.

Material and Methods.

It was thought best to carry on the experiments in wound healing with the frog inasmuch as the tissues used in the previous work with living tissue cultures had been obtained from this animal. During the present experiments thirty-one animals were used and a total of fifty operations were performed. The process of wound healing has been studied at various stages both in the living animals and in the preserved material. For the study of the preserved material pieces of the tissues containing the wounds were removed at various times after the operations. These were preserved in Zenker's solution, imbedded in paraffin, and sections, in various planes, were made at 10 μ. In general the stain used has been Mallory's connective tissue stain modified according to Mall.[3] Mallory's stain, unmodified, and the Van Gieson picro-fuchsin stain have also been used for comparison. A discussion of the staining reactions will be found in a later section of this paper.

In the preliminary experiments, the semimembranosus muscle lying on the dorsal surface of the hind legs of the frog was used. After anesthetization the animal was placed on an operating board dorsal surface up. The skin was then sterilized by washing it with a 0.001 per cent solution of mercuric chloride. This method of sterilization appears to be adequate for this work and there has been very little trouble from infection. An incision of about 5 to 7 mm. in length was then made in the median dorsal surface of either or both of the hind legs. The cut edges of the skin were separated and held apart and a cube of the underlying muscle tissue measuring about 2 mm. was removed. The cavity thus formed in the muscle tissue was either filled with plasma which had been obtained in the usual manner[4] from another frog, or by the blood and lymph which flowed into the wound from the cut edges. After the operation the frog was held in position for a few minutes until the clot had formed in the wound.

The results obtained from these experiments were not satisfactory. The many movements of the legs and the consequent expansion and contraction of the muscle fibers resulted, in many cases, in a breaking up of the plasma clot in the wound cavity and its destruction, so that it was impossible to make a study of it. The friction of the overlying skin of the leg during these movements also tended to make the operations unsuitable for the present study of the fibrin clot. In a number of these experiments which were first made, stitches were taken in the skin to hold the cut edges together and in their proper positions. In the later operations it was found that this was not necessary for it was observed that when a cut was made in the skin, as noted above, if the animal remained quiet for a few minutes after the operation, the coagulation of the blood and lymph formed a tissue of sufficient strength to hold the cut edges of the skin in their proper position. It appeared evident from this that wounds in the frog skin would afford a suitable field for the study of the process of wound healing, particularly with regard to the action and fate of the fibrin clot. Later experiments showed that the skin lying on the posterior dorsal surface of the animal offered an even better field for these experiments than did the skin of the legs inasmuch as it is not subject to an almost continual, and at times violent strain as the result of leg movements. Wounds in both these regions have been studied and form the basis of the results given in the present paper.

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[3] Mall, F. P., On the Development of the Connective Tissues from the Connective-Tissue Syncytium, *Am. Jour. Anat.*, 1901–02, i, 338.

[4] Baitsell, G. A., The Origin and Structure of a Fibrous Tissue Which Appears in Living Cultures of Adult Frog Tissues, *Jour. Exper. Med.*, 1915, xxi, 456.

Observations on the Process of Wound Healing in the Skin of Living Frogs.

The healing of various types of wounds has been observed in the living animals. In the first type of experiments, a simple incision was made in the skin of the frog either on the dorsal surface of the leg or on the back. As far as could be told by the observations on the living animal these wounds healed very quickly. The formation of the coagulation tissue as a result of the clotting of the blood plasma or lymph present in the wound occurred within a few minutes after the wound was made. As a result the cut edges were firmly held in position and the frog could then be placed in the aquarium without injury to the new tissue. The animals examined 24 hours later showed only slight evidence of the wound. The cut edges were still firmly held and all that could be noted was the light colored line of coagulation tissue marking the extent of the incision. In the course of the next day or so the epithelial cells moved over this area and obliterated all evidences of the wound.

In the second type of experiments a piece of skin measuring about 5 by 2 mm. was detached on three sides. The fourth side (2 mm.) was left attached to the body. In this way a flap of skin was formed which was severed from the body except at one end. In such cases the coagulation tissue which quickly formed held all the cut edges of the skin and the loose flap in place, and thus an operation which one would naturally expect, without the aid of sutures to hold the edges in place, to have left a large open wound in the skin with a consequent slow healing was permanently closed within a few minutes after the operation. In the following days one could observe, as in the previous type of experiments, a gradual obliteration of the cuts by the epithelial cells which moved out over the coagulation tissue from the cut edges of the skin.

In the third type of experiments a piece of skin from the median posterior region of the back, measuring about 5 mm. square, was completely severed from the body of the animal, placed in sterile Ringer solution for a few minutes, and then replaced in the cavity from which it had previously been taken. The observations showed that no other support than the coagulation tissue which formed was necessary in order to hold such a piece of skin in position in the wound cavity, and the complete obliteration of the wound occurred in a few days by the movement of the epithelial cells over the coagulation tissue. The results of other experiments of this type showed that the living skin from another animal or a dead tissue, such as the stratum compactum obtained by pancreatin digestion of adult frog skin, could be transplanted into a wound cavity in the skin and retained there by the action of the coagulation tissue alone. In a later paper it is hoped that more complete results regarding such skin transplantation can be presented. The only fact regarding them which should be emphasized in the present paper is the action of the coagulation tissue in the wound cavities as shown by its ability to hold the transplant in position without the aid of sutures.

In the last type of experiments, a piece of skin, varying in size from 3 to 5 mm. square, was completely removed from the body. The resulting cavity was quickly filled with the coagulation tissue. In this type of experiment it was found that in some cases, particularly if the cavity caused by the removal of a piece of skin was large, it was better to supply additional plasma obtained from another animal in order that the wound cavity might be completely filled. A wound of this type (Fig. 1) gives the best opportunity for the study of the coagulation tissue. It forms a hyaline mass in the wound cavity and is glossy and transparent, and until it has been covered by the epithelial cells which move in from the surrounding areas the underlying muscle tissue can be seen through it. This fibrin clot or coagulation tissue when it is first formed may sometimes be destroyed by extremely violent movements of the animal, especially if the wound be a large one. An increase in the strength of the new tissue soon becomes evident and in a few days it appears to be as immune from injury as regular skin tissue. As far as can be told by the observations on the living animals, this coagulation tissue formed by the clotting of the plasma in the wound remains permanently and takes the place of the piece of skin tissue which was removed. From this it is clear that in a skin wound in the frog, if the conditions are right, a new tissue can be formed almost immediately after the injury which is sufficiently strong to retain its place in the wound cavity and to hold the cut edges in position. The wound, to all appearances, is thus healed without waiting for a process of regeneration of tissues by cell division. Probably the most impressive part of this process of wound healing, to an observer, is the rapidity with which it takes place. The coagulation of the plasma closes the wound almost immediately and then, evidently by a transformation of this fibrin clot, a firm, resistant, and apparently permanent tissue is formed which serves as a regular connective tissue.

During the course of the experiments, observations have also been made on the healing of wounds in which no coagulation tissue was present. Such a condition may arise either because of the lack of a sufficient amount of plasma in the wound at the time it was made, because of a later destruction of the coagulation tissue as a result of tension caused by too vigorous movements, or because of an infection. In cases of this kind the process of wound healing in the skin of a frog is a comparatively long and slow one, and in some cases results in the death of the animal by a later infection received through the open wound.

Study of the Prepared Material.

In Fig. 1 a view of a wound in the skin of a frog is shown *in toto* at a magnification of about 15 diameters. This wound was removed from the animal and preserved when it was three days old. The wound, caused by the removal of a piece of skin, originally measured about 5 by 2 mm. The figure was drawn with the aid of a Zeiss binocular microscope from the under surface of the skin and shows the wound surrounded by the skin tissue and filled with the coagulation tissue (fibrin clot) which was formed within a few minutes after the wound was made by a clotting of the blood plasma and lymph. Several large blood vessels are shown, some of which run into the coagulation tissue. Some of these vessels appear to be enlargements of the vessels supplying the skin in that region, while others apparently arise in the underlying muscles. In some cases the wound is found to be attached to the muscles by a number of small vessels.

A transverse section of the same preparation is shown in Fig. 2 at a magnification of 77 diameters. At either end of this figure may be noted the cut ends of the skin which mark the boundaries of the wound. Connecting these can be seen the bridge of new tissue which has formed in the wound cavity. Lying above this coagulation tissue are several layers of epithelial cells which have wandered in from the skin tissue surrounding the wound. This movement of the epithelial cells begins very soon after the wound has been made and continues until, as shown in later stages (Fig. 6), they form a thick layer over the coagulation tissue. The epithelial cells in Fig. 2 are more numerous near the cut edges of the skin. At these points the coagulation tissue is drawn out into a rather thin strand which is attached to the cut edges of the skin and which serves to hold them in place. It can be noted in this figure that fibers are present in the coagulation tissue.

The observations on the living animals have shown that the coagulation tissue in a 3 day wound is very firm and resistant as compared with a young fibrin clot. From this it is evident that already some change has taken place in the clot and this fact can also be noted from the study of the prepared material. In Fig. 3 a portion of the coagulation tissue, from the same preparation as Fig. 2, is shown at a magnification of 787 diameters. At this magnification one is able to see clearly the histological structure of the coagulation tissue. Quite large areas can be noted in this figure in which the fibrin net has not been changed but still retains its characteristic structure. In other regions, however, the fibrin net has been changed into a new fibrous tissue. In such areas it appears that there has been a fusion of the elements of the fibrin net to form long, wavy, fibrous bundles which are typical in structure and appearance to those found in various forms of connective tissues. This new fibrous tissue is apparently identical with that which forms in plasma clots in tissue cultures. The transformation of the fibrin clot in this wound into a new fibrous tissue has not been due to an intracellular action for, with the exception of some blood corpuscles which were present in the plasma when it clotted, some of which can be seen in Fig. 3, no cells of any kind are present in the new fibrous tissue except in the extreme edges where the new tissue is in contact with the cut edges of the skin. A thorough study of this point has been made in this and other similar preparations and it can be definitely stated that in wounds in the frog skin, as has previously been shown to be the case in tissue cultures, a direct transformation of a fibrin clot into a new fibrous tissue will occur without intracellular action.

In Fig. 4, which is a portion of a longitudinal section of a 4 day wound at a magnification of 787 diameters, a more complete transformation of the plasma clot is seen. The section from which this figure was drawn was taken from near the upper surface of the coagulation tissue; that is, from the dorsal portion of the clot lying just below the epithelial cells. At either edge of the coagulation tissue may be noted some of the epithelial cells which mark the boundary of the wound and which have grown down on both sides of the coagulation tissue. This downward growth of the epithelial cells can be noted in some of the transverse sections of the wound tissue (Fig. 2). A study of Fig. 4 shows that the fibrin net structure has disappeared and the new fibrous tissue has been formed which, as in Fig. 3, is composed of bands of fibrils showing the typical structure of regular connective tissue fibers. The new fibrous tissue is denser near the bottom of the figure and it is in this region that its structure is seen to the best advantage. All the coagulation tissue shown in the figure has lost the original fibrin net structure, but in the less dense regions of this tissue, in which numerous vacuoles are present, the transformation into the new fibrous tissue has not been so complete. No fibroblast cells are present in this region of the clot and so in this case also the transformation of the fibrin clot into the new fibrous tissue has not been due to any intracellular action. In this preparation centrifuged blood plasma which had been obtained from another animal was placed in the wound to form the clot and this fact accounts for the almost complete absence of blood corpuscles. Those that are shown in this figure are in most cases matted together and are evidently undergoing degeneration.

In Fig. 5 is shown, at the same magnification as Fig. 4, another longitudinal section of the same preparation taken at a deeper level. In this figure a considerable number of both fibroblast cells and blood corpuscles are shown. In this figure a transformation of the fibrin clot has also occurred and a new fibrous tissue has been formed containing bundles of wavy fibers. These are particularly well shown to the left of the figure. In this region numerous cells are also present and as a result the bundles of fibers are more or less separated from each other. To the right of the figure fewer cells are present and the newly formed fibrous tissue is more closely massed together. The bundles of fibers, however, can be clearly seen.

A comparison of the new fibrous tissue shown in Figs. 3, 4, and 5, which has been formed through a transformation of the fibrin clot, with the fibrous tissue found in the plasma clot in living cultures of frog tissues, as noted in a previous paper,[5] shows that they are identical.

The conditions present in an older wound are shown in Fig. 6, which is a transverse section of a 12 to 13 day preparation at a magnification of 240 diameters. At either end of the figure can be seen the cut ends of the skin tissue which mark the boundaries of the wound and between these the new tissue which has been formed in the wound. Overlying this region are the epithelial cells which are piled up many layers thick. The wound cavity, except for a small portion lying near the right end of the figure, is filled with the new fibrous tissue. Fibroblast cells are present all through the new tissue. In the region lying to the right a portion of the original fibrin clot apparently still remains. In Fig. 7, which was drawn from the same section as Fig. 6, this region of the wound is shown at a magnification of 787 diameters. The figure shows that the fibrin clot has lost its original structure, as shown for example in Fig. 3, and has become a more or less compact mass in which, at various places, it can be noted that fibers have formed. Around the edges of the fibrin mass, in particular, a great many of the bundles of fibers can be seen. These are attached to the fibrin clot and it is clear that they have arisen by a transformation of its elements and not from an intracellular action. The cells which are present stand out distinctly from the fibers, as shown in the figure, and show no connection with them.

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[5] Baitsell, G. A., The Origin and Structure of a Fibrous Tissue Which Appears in Living Cultures of Adult Frog Tissues, *Jour. Exper. Med.*, 1915, xxi, 478–79. Compare Fig. 3 of this paper with Figs. 11 and 17 of previous paper.

In Fig. 8 is shown, at a magnification of 787 diameters, a longitudinal section of another wound which is practically the same age as the one shown in Figs. 6 and 7. In Fig. 8, however, we find that the formation of the new tissue has proceeded more rapidly and consequently the original fibrin net has been entirely transformed into the new fibrous tissue which fills the wound cavity. Blood vessels are forming in various regions in this new tissue and numerous cells, which for the most part show the typical spindle shape of fibroblast cells, are present but stand out clearly and distinctly from the fibers. The question of the relations existing between the new fibrous tissue and the cells which wander into it from the surrounding edges of the skin is one which has been studied with care. It has already been pointed out that the transformation of the fibrin clot occurs previous to the appearance of any cells, so that it cannot be due to an intracellular action (Figs. 3 and 4). A study of the preparations shows that the cells when they first appear in the new fibrous tissue are round in shape (Figs. 5 and 7). Later they elongate and assume the typical spindle shape of fibroblast cells (Fig. 8). This change in the shape is apparently due to the stereotropic action of the cells in moving along the fibers.[6] The movement of the cells in the newly formed fibrous tissue appears to result in a separation of the bundles of fibers and we have as a result what, to all appearances, is a typical scar tissue. With the Mallory stain the cells are colored yellow and they are distinctly differentiated from the fibers of the new tissue which are colored blue by the stain. The preparations do not show any evidence of a digestion by the cells of the new fibrous tissue or of any attempt by them to form new fibers intracellularly. The condition shown in Fig. 8 is typical of the condition found in much older wounds (3 to 4 weeks) and the evidence appears clear from these results that, in the healing of skin wounds in the frog, the new fibrous tissue present in the wound which has been formed by a direct transformation of the fibrin clot remains as the permanent connective tissue.

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[6] Harrison, R. G., The Reaction of Embryonic Cells to Solid Structures, *Jour. Exper. Zool.*, 1914, xvii, 521.

Nature of the New Fibrous Tissue.

The appearance, structure, and staining reaction with Mallory's stain of the new fibrous tissue are, as shown in the previous paper, identical with those of regularly formed connective tissues. In an endeavor to settle definitely the real relationship existing between regular frog connective tissue and the new fibrous tissue several series of tests have been made.

Staining Reactions of the Fibrous Tissue Formed in Wounds.—The Mallory connective tissue stain, either modified or unmodified, has proved to be more specific in its reaction than any other stain that has thus far been used in the work, although various other methods have been tried. This stain will color connective tissue fibers of the frog an almost perfect ultramarine blue if they are not too closely packed together. If, for example, a piece of skin from an adult frog is stained with Mallory's stain it will be found, in general, that the stratum spongiosum, in which the bundles of connective tissue fibers are loosely packed together and in which the individual fibrils can be distinguished, will be colored an ultramarine blue. On the other hand, in the stratum compactum of the same preparation in which the bundles of fibers are closely massed, it will be found that this layer will be stained a color varying from yellow to bright red, depending upon the manipulation of the stain.

The new fibrous tissue formed in the clots shows the same color reaction to a Mallory stain as does the connective tissue in the skin. When the bundles of fibers in the new fibrous tissue are not closely matted together, as shown for example in Figs. 3, 4, and 5, the Mallory stain gives them a typical blue color as in the stratum spongiosum of the skin. On the other hand, when the new fibrous tissue is very compact and the individual fibrils composing the bundles cannot be distinguished, then, in many cases, the result will be a color varying from yellow to red, similar to that found in the stratum compactum of the skin. The same color also generally results from the Mallory stain in very young preparations in which the clot has not been changed into the new fibrous tissue but retains its original structure.

With a Van Gieson picro-fuchsin stain the new fibrous tissue shows a negative reaction. This stain, however, does not appear to be a specific one for connective tissues of the frog. The results show that it acts in just the opposite way that Mallory's stain does, in that it only gives the characteristic red color for connective tissues when a heavy tissue is present in which the bundles of fibers are closely massed together. In the frog skin, for example, the stratum compactum occasionally stains red, whereas in the stratum spongiosum an entirely negative reaction is obtained. In most cases, however, all the connective tissues of the frog skin as well as those from other parts of the body give a negative color reaction with this stain. In this connection a series of staining tests were made with embryonic connective tissue from the tail of a 50 mm. tadpole. It consists of a mass of connective tissue fibers loosely packed together. These fibers will take a typical stain with Mallory's stain, but with Van Gieson's the reaction is negative, as it is with the new fibrous tissue. The results in brief from all these experiments show that the Van Gieson's stain is not specific for connective tissue fibers in the frog whether embryonic or adult, and the same is true for its reaction with the new fibrous tissue.

Digestion Tests of the Fibrous Tissue Formed in Wounds.—Of all the tests used to distinguish between fibrin and connective tissues, the digestion tests are regarded as being the most specific and conclusive. A young fibrin clot is easily digested in a pancreatin solution whereas connective tissues resist the action. In the previous paper it was noted that the transformed fibrin net in the tissue cultures was easily dissolved in pancreatin, and this fact gave evidence that the new fibrous tissue was fibrin in character although greatly changed in appearance from the typical fibrin net. However, it was also pointed out that in this case the test was not conclusive, inasmuch as the newly formed fibers in the clot were imbedded in a fibrin net which was not completely transformed, and the digestion of the unchanged fibrin clot by the pancreatin would naturally cause a breaking down and scattering of the fibers which had formed in it, and remained connected with it. In other words, the surrounding unchanged fibrin constituted the sole support of the fibers which had formed from a part of it.[7] This close relation existing between the fibrin clot and the new fibrous tissue also prevents the digestion tests being used in the present experiments to furnish a conclusive answer as to the real nature of the transformed fibrin net tissue in the clots formed in wound healing.

In this connection the results secured by a series of experiments in which the embryonic connective tissues of the tadpole were subjected to pancreatin digestion should be noted. In these experiments pieces of skin, taken from tadpoles ranging in length from 7 mm. to large tadpoles of about 70 mm. just before metamorphosis, were placed in pancreatin solutions of uniform strength[8] and digested over night at 38°C. In all these experiments a complete digestion of the tadpole skin occurred. This same result was obtained when the connective tissue from the tails of tadpoles of various ages was digested in pancreatin solutions. These young fibers, as has been noted, stain typically with Mallory's stain and are apparently fully formed. Nevertheless they can be completely digested in pancreatin in a few hours. These results with embryonic connective tissues are entirely different from those obtained when connective tissues from an adult frog are subjected to the same treatment, for the experiments show that the mature connective tissues in the skin of an adult frog are able to resist the action of the pancreatin and remain as a mass of tissue which, as noted above, could be used in the implantation experiments.

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[7] Baitsell, G. A., The Origin and Structure of a Fibrous Tissue Which Appears in Living Cultures of Adult Frog Tissues, *Jour. Exper. Med.*, 1915, xxi, 473.

[8] The pancreatin extract used in all these digestion experiments was obtained by the well known Roberts' method. The author is indebted to Professor F. P. Underhill for his assistance in this matter.

These results demonstrate that the reaction of the embryonic connective tissues of the frog to pancreatin digestion is the opposite of that of adult connective tissue.

The results of both the staining and digestion tests, in brief, show that with these methods one is unable to differentiate between the new fibrous tissue, formed by a transformation of the fibrin clots, and embryonic connective tissues of the tadpole, and that the connective tissues of the adult, while reacting in the same way to the stains, can be differentiated from both the new fibrous tissue and embryonic connective tissue by their ability to resist the pancreatin digestion.

There is the possibility that the same resistance to pancreatin digestion which must develop in embryonic connective tissue as it becomes mature, as shown by the digestion tests on the mature and embryonic connective tissues, might also develop in time in the new fibrous tissue which has resulted through a transformation of the fibrin clot, for it is well known that old fibrin clots become highly resistant to various agents which will entirely dissolve younger clots. Mall[9] says: "Fresh fibrin is easily dissolved in dilute acid or digested in pancreatin. After fibrin becomes older, as in fibrinous deposits, the fibrils become very resistant and are not dissolved in boiling acetic acid, 20 per cent, in cold concentrated KOH, H_2SO_4, HCl, HNO_3, or nitro-hydrochloric acid." What changes take place in these fibrinous deposits that cause such an increase in their resisting powers is apparently not known. From the present results one would naturally suggest that in such cases, in the course of time, a complete transformation of the fibrin clot into the new fibrous tissue had occurred and that this transformed tissue, when old enough, was able to resist the action of pancreatin and various other destructive agents.

This point has proven to be one which is difficult of solution. The fact that even the fully formed connective tissue of large tadpoles is unable to resist the pancreatin digestion makes it impossible to expect that one could ever keep tissue cultures alive long enough to secure a new fibrous tissue from the fibrin clot which would be able to resist the pancreatin digestion and thus show definitely that it could not be distinguished from the regular connective tissues in this regard. In wounds the problem is complicated by the presence of the cells which wander into them and through the new fibrous tissue soon after it has been formed from the fibrin clot. The preparations, as stated above, do not show any evidence of an attempt by these cells to form new fibers intracellularly, but, nevertheless, their presence in the wound for the long period of time which evidently is necessary for the new fibrous tissue to become resistant, makes the problem a difficult one.

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[9] Mall, F. P., Reticulated Tissue, and Its Relation to the Connective Tissue Fibrils, *Johns Hopkins Hosp. Rep.*, 1896, i, 182, 194.

DISCUSSION.

The experiments reported in the present paper demonstrate that in wounds made in the skin of adult frogs there occurs, as has previously been shown to be the case in living cultures of adult frog tissues, a direct transformation of the fibrin clot into a new fibrous tissue without any intracellular action. This newly formed fibrous tissue which fills the wound space is apparently identical in appearance, structure, function, and staining reactions with regularly formed permanent connective tissue. It differs from adult connective tissue in the skin of the frog in its reaction with pancreatin digestion. However, this test, as well as all others that have so far been tried, fails to differentiate between the new fibrous tissue and young connective tissue found in tadpoles of various stages.

Two views might be held with reference to the fate of this new fibrous tissue directly formed from the fibrin clot. The first is that it is only a temporary tissue and that it will be replaced in time by a permanent connective tissue formed through an intracellular action of the fibroblasts. This is in accord with the generally prevailing views[10] held at present, which are that the fibrin, in wounds healing by first intention, forms a temporary tissue which holds the wound surfaces in place and stops up the wound thus preventing infection. Later the fibrin disappears, not by liquefaction, but through an actual consumption by the new tissue cells which show a positive chemotropism to the fibrin mass and are thus attracted into the fibrin clot in large numbers. The permanent tissues which go to fill the wound space are then formed by these cells through an intracellular action.

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[10] For a general discussion and summary see Marchand, F., Der Process der Wundheilung, Stuttgart, 1901, 52–55.

The other view is that this new fibrous tissue remains in the wound as a permanent connective tissue. That is to say, at least in wound healing in the frog skin, a reaction occurs which is able to transform a fibrin clot by a direct intercellular action into a permanent fibrous connective tissue. This view is supported by the work of Hertzler[11] who finds that, in experimental peritoneal adhesions in the dog, a new fibrous tissue, which he believes remains permanently, is formed by a transformation of a fibrin net. Speaking of the formation of this fibrous tissue, he says:[12] "My researches have convinced me that the cell is not primarily the active agent, but that the initial processes are chemical and are identical with those of blood coagulation, the cell playing an entirely secondary rôle."

The results obtained in the present experiments also give evidence in favor of the latter view. The fibroblast cells which wander into the new fibrous tissue apparently do not digest the fibers which have previously been formed, nor is there any evidence revealed, by a thorough study of the preparations, of an attempt by these cells to form new fibers intracellularly. Their only apparent action is to break up the larger bundles of fibers by their movements among them. In a wound of about 12 days a typical scar tissue is generally present (Fig. 8), and later stages (3 to 4 weeks) do not show any further changes in the structure of the connective tissue present in the wound.

SUMMARY.

1. In experimental wounds, made by removing various sized pieces of skin from the frog, there is a rapid coagulation of the blood plasma and lymph to form a coagulation tissue which fills the wound cavity.
2. The observations on the living animals show that the coagulation

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[11] Hertzler, A. E., Pseudoperitoneum, Varicosity of the Peritoneum and Sclerosis of the Mesentery. With a Preliminary Note on the Development of Fibrous Tissue, *Jour. Am. Med. Assn.*, 1910, liv, 351; The Development of Fibrous Tissues in Peritoneal Adhesions, *Anat. Rec.*, 1915, ix, 83.

[12] Hertzler, A. E., Pseudoperitoneum, Varicosity of the Peritoneum and Sclerosis of the Mesentery. With a Preliminary Note on the Development of Fibrous Tissue, *Jour. Am. Med. Assn.*, 1910, liv, 352.

tissue becomes more and more resistant and is generally of sufficient strength to hold the cut edges of the wound in place and to retain its position in the wound cavity. It serves, at least temporarily, as a connective tissue and as a base for the epithelial cells which rapidly move in from all the cut edges and cover the wound.

3. The study of the prepared sections of wound tissue show that at first in the coagulation tissue, formed as a result of the clotting of blood and lymph, a typical fibrin net is present in the wound (Fig. 3). Later this fibrin net is transformed into a new fibrous tissue containing bundles of wavy fibers in which, in many instances, the individual fibrils can be noted (Fig. 4). This transformation of the clot and the formation of the new fibrous tissue takes place before the tissue cells wander into the coagulation tissue and therefore cannot be due to an intracellular action. It is a direct transformation of the fibrin clot and is identical with the process which was previously found to take place in the fibrin clots in living cultures of adult frog tissues.

4. The tissue cells, which later move into the new fibrous tissue in large numbers from the surrounding areas, do not digest the fibers but, apparently by their movements, cause a division of the large bundles into smaller ones (Fig. 5). These cells when they first appear in the fibrous tissue are rounded, but later they assume the typical elongated spindle shape of fibroblast cells (Fig. 8). The preparations do not show any connection between these spindle-shaped cells and the fibers which had already formed, nor is there any evidence of a later attempt by them to form new fibers intracellularly.

5. The staining reactions of the new fibrous tissue appear to be identical with the staining reactions of the connective tissue in frog skin. However, the new tissue can be digested in pancreatin and in this reaction it differs from the connective tissue in the skin of the adult frog. On the other hand, extensive experiments with pancreatin on embryonic but fully formed connective tissue, obtained from the tail and skin of tadpoles of various ages, show that pancreatin will digest it just as it does the newly formed fibrous tissue.

EXPLANATION OF PLATES.

All the figures, with the exception of Fig. 1, were drawn from prepared material which had been preserved in Zenker's solution, sectioned at 10 μ, and stained with Mallory's connective tissue stain.

PLATE 106.

FIG. 1. 3 day wound from back of frog, shown *in toto* from the under surface. × 15. A piece of skin measuring about 5 by 2 mm. was removed and the cavity thus formed is shown filled with the coagulation tissue (C.T.). A considerable area of the skin tissue (SK.T.) surrounding the wound is shown, and blood vessels (B.V.) are also present.

FIG. 2. Transverse section of the preparation shown in Fig. 1. × 77. Cut edges of the skin tissue (SK.T.) marking boundaries of the wound are shown at both ends of the figure. These are connected by the coagulation tissue (C.T.) in which fibers (FIB.) can be noted at various places. Epithelial cells (EP.C.) which have moved in from the surrounding areas overlie the coagulation tissue. Section of blood vessel (B.V.) and numerous blood corpuscles (B.C.) are also shown.

PLATE 107.

FIG. 3. Transverse section showing a portion of the coagulation tissue from the same preparation as Figs. 1 and 2. × 787. The figure shows the structure of the coagulation tissue present in a 3 day wound. Some of this tissue retains the fibrin net (F.N.) structure of the original fibrin clot. Other areas show new fibrous tissue (N.F.T.) which has been formed by a transformation of the elements of the fibrin net. Numerous blood corpuscles (B.C.) are present, but no fibroblast cells.

FIG. 4. Longitudinal section of a 4 day wound in the leg of a frog. × 787. The coagulation tissue has been transformed into the new fibrous tissue (N.F.T.) in which bundles of wavy fibers can be seen. Transformation is most complete in the denser region of coagulation tissue lying to the right of the figure. No fibroblast cells are present, but a few blood corpuscles (B.C.) are shown which are evidently undergoing degeneration.

FIG. 5. Longitudinal section from the preparation shown in Fig. 4. × 787. The section from which this drawing was made was taken at a deeper level of the wound than Fig. 4; *i.e.*, just above the underlying muscle tissue. To the right the new fibrous tissue (N.F.T.) is more dense, but even here the fibrous structure can be seen. To the left it is broken up into wavy fibrous bundles among which numerous cells (FBL.C.) are appearing. Blood corpuscles (B.C.) are also present.

PLATE 108.

FIG. 6. Transverse section of a 12 to 13 day wound preparation. × 240. Cut ends of the skin tissue (SK.T.) are shown at the right and left of the figure.

The wound cavity is almost filled with the new fibrous tissue (N.F.T.) except for a small portion of fibrin clot (F.C.) lying to the right. Epithelial cells (EP.C.) are piled up many layers thick above the new tissue. Cells (FBL.C.) are also present in the new tissue. Compare Fig. 7.

FIG. 7. A portion of the preparation shown in Fig. 6. × 787. This drawing shows the remains of a fibrin clot (F.C.) lying to the right in Fig. 6. It has lost its original fibrin net structure and has become a more or less compact mass. Numerous fibers can be seen running through it, and around the edges are a great many fibers which have resulted from a transformation of the fibrin clot and which go to form the new fibrous tissue (N.F.T.). Numerous rounded cells (FBL.C.) are present, some of which are beginning to assume the typical spindle shape of fibroblast cells. They stand out clearly and show no connection with the fibers.

PLATE 109.

FIG. 8. Longitudinal section of another 12 to 13 day wound preparation. × 787. This figure shows complete transformation of coagulation tissue into new fibrous tissue (N.F.T.) which fills the wound cavity between the cut edges of the skin tissue (SK.T.). Blood vessels (B.V.) are forming and numerous spindle-shaped fibroblast cells (FBL.C.) are present. These cells stretch out along the fibers which had previously been formed through a transformation of the fibrin net and apparently separate the bundles. They do not digest the fibers nor is there any evidence of an attempt by them to form new fibers intracellularly.

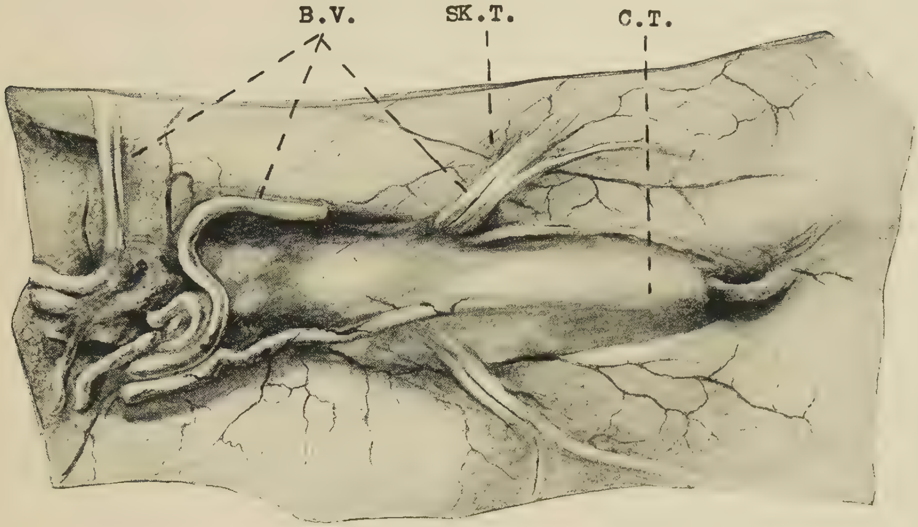


Fig. 1.

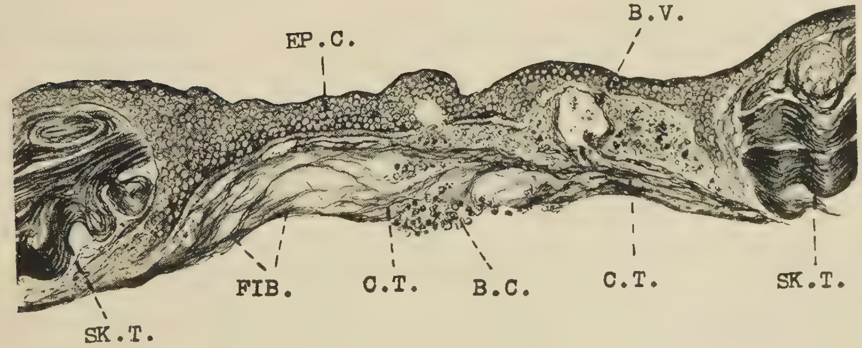


Fig. 2.

(Baitsell: Fibrous Tissue Formed in Wound Healing.)

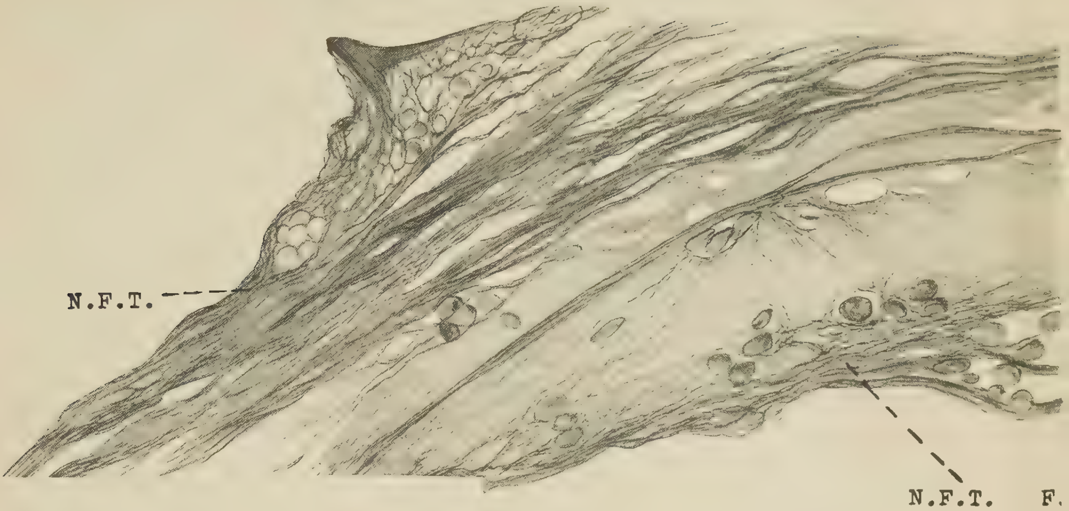


FIG. 3.

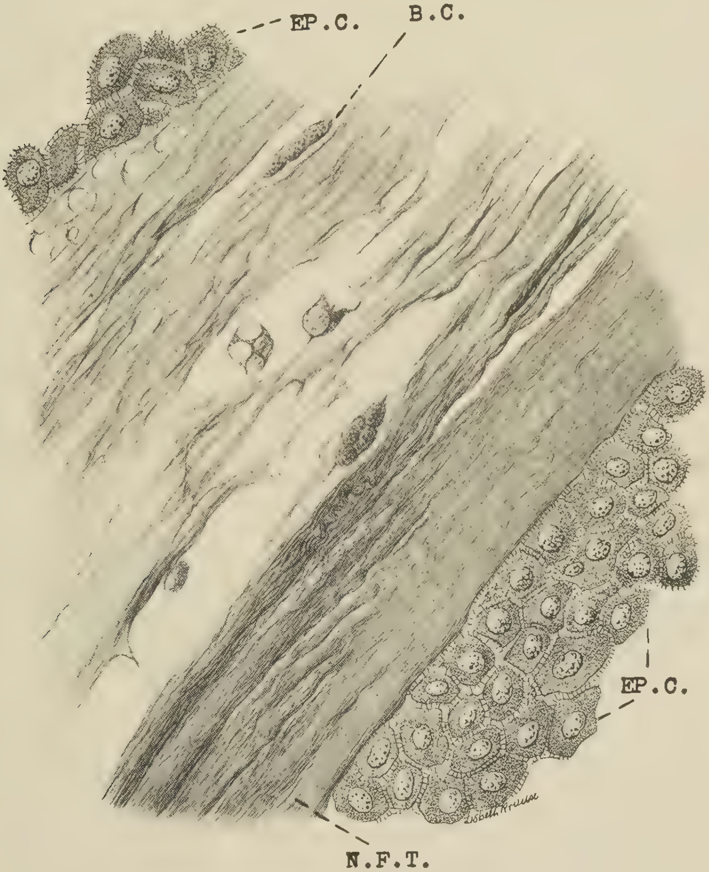
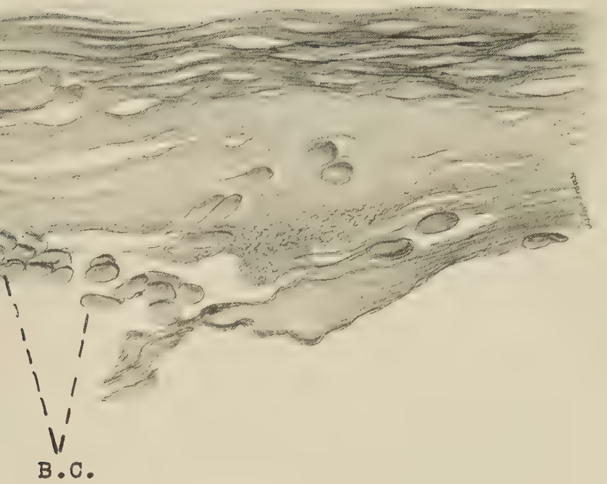


FIG. 4.



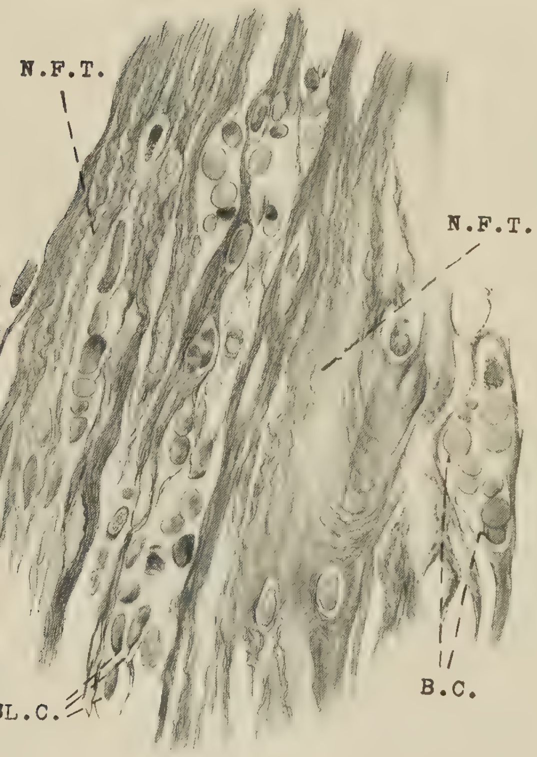


Fig. 5.

(Baitsell: Fibrous Tissue Formed in Wound Healing.)

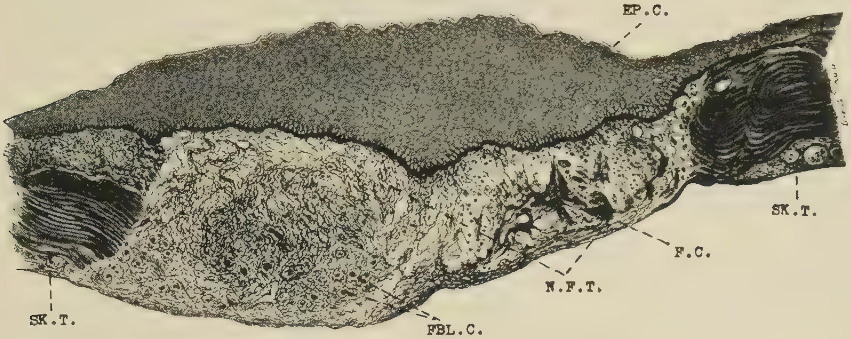


FIG. 6.

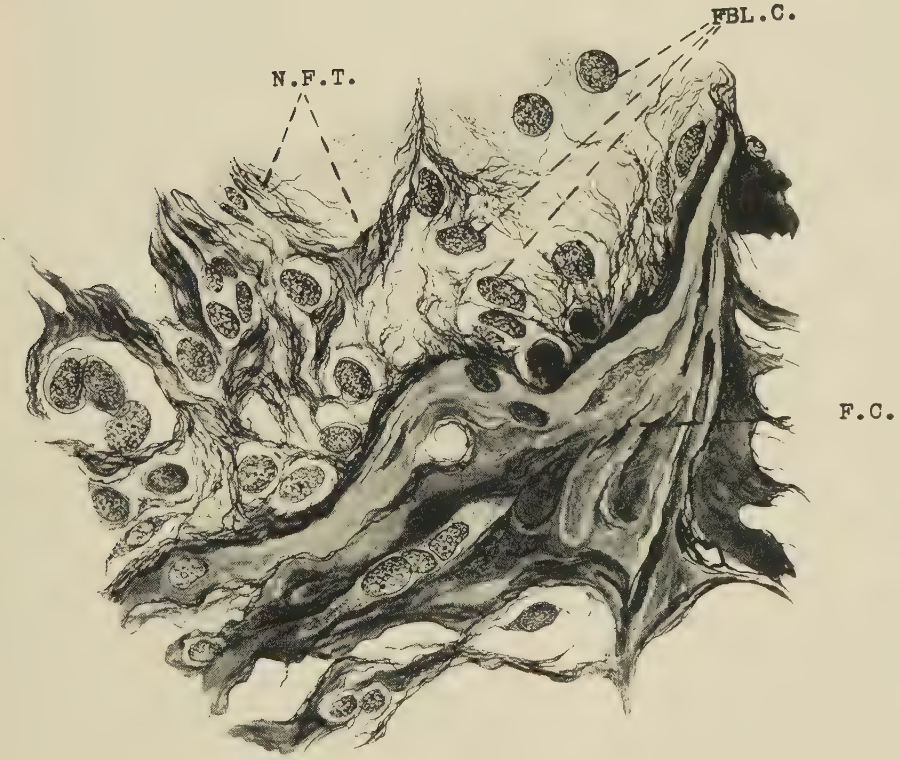


FIG. 7.

(Baitsell: Fibrous Tissue Formed in Wound Healing.)

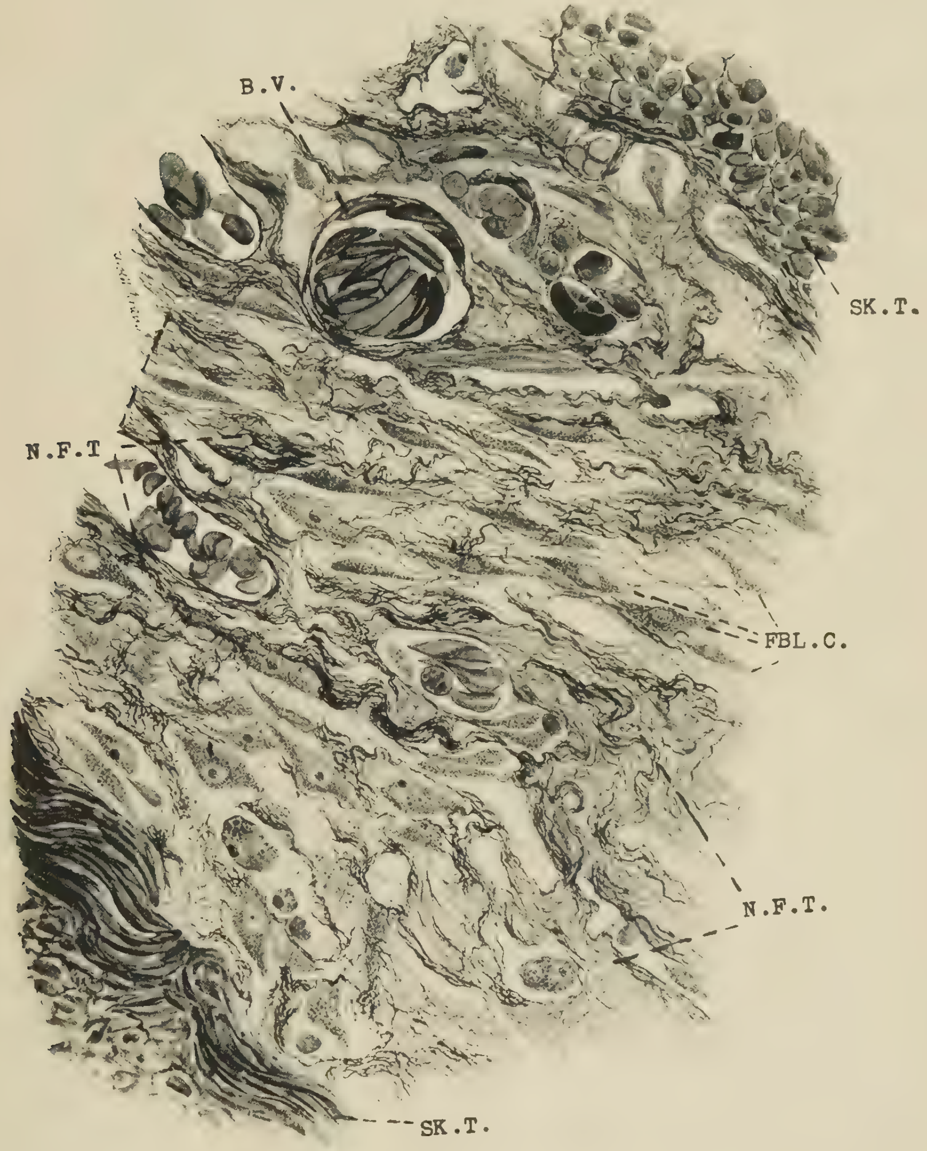


FIG. 8.

(Baitsell: Fibrous Tissue Formed in Wound Healing.)

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THE DEVELOPMENT OF PARAVORTEX GEMELLIPARA

(GRAFFILLA GEMELLIPARA LINTON)

STANLEY C. BALL

Department of Zoology, Yale University

SIXTEEN TEXT FIGURES AND NINE PLATES

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I. INTRODUCTION

1. Determination of the species

In the Brooks Memorial Volume of The Journal of Experimental Zoology, vol. 9, 1910, Prof. Edwin Linton described a new species of Rhabdocoele Turbellarian which he had discovered living in the ribbed mussel, Modiolus demissus. He referred it with some hesitation to the genus Graffilla (Jhering). Von Jhering ('80) in establishing the genus Graffilla characterized it in the following words:

> Pharynx nach Art des Mesostomeenschlundes aus Muskelfasern und eingelagerten Bindegewebszellen bestehend, nicht in einer Tasche gelegen, sondern direkt mit dem Munde zusammenhängend. Bursa copulatrix und Receptaculum seminis durch ein Organ vertreten. Ovarien langgestreckt, bandfömig, Dotterstöcke sehr reich verästelt.

Most important in the determination of the genus in which Linton's Turbellarian is to be placed is the question of the receptaculum seminis. Von Jhering in his figures of Graffilla muricicola indicates that two reservoirs are connected with the atrium (called by him the 'uterus'). The more anterior of these is the vesticulum seminalis which bears a short conical penis on its lower surface and projecting into the atrium. The receptaculum seminis lies just posterior to the seminal vesicle, and likewise dorsal to the atrium. It s to be noted that the latter is not merely a dorsal lobe of the atrium but is a distinct spherical reservoir, fully as large as the seminal vesicle, and connected with the atrium by a slender duct of a length about equal to its own diameter. This canal as described by von Graff for Graffilla buccinicola is shorter but none the less definite. It is therefore characteristic of the genus Graffilla that the seminal receptacle is not sessile upon the atrium but connected with it by a canal. An examination of the Turbellarian discovered by Linton reveals no such reservoir.

This American species may be described as a Vorticid Rhabdocoele occasionally reaching 2 mm. in length and 0.80 mm. in diameter at its widest part; elongated ovoid in form while at rest, young specimens nearly linear when moving rapidly, posterior half of body somewhat broader, anterior quarter more flattened than central and posterior portion which is nearly cylindrical; ends bluntly pointed, the posterior often being the more acute; white with yellowish tinge, semi-transparent; body contractile and extremely flexible; crawls on bottom sometimes in a straight line, often performing circus movements; sometimes swims freely in water, revolving about its longitudinal axis and so follows a spiral course; pharynx sub-globose (doliiform) opening anteriorly into a small vestibule leading to the mouth; the latter is ventral but so close to the anterior end that it is directed forward; oesophagus not distinct from the spindle-shaped intestine into which it merges; intestine extends nearly to the posterior end, lies in upper half of body, walls thick, composed of large cells elongated toward the lumen; two black reniform eyes above the pharynx and anterior to the well developed two-lobed brain. Genital pore ventral at the end of the anterior third of the body, opening into a large atrium; prominent pear-shaped seminal vesicle carries at its lower end a muscular penis (without chitinous parts) which lies in a very small atrium masculinum; atrium produced posteriorly and dorsally into a pouch which acts as a bursa copulatrix; distensible antrum femininum extends posteriorly from the middle of the posterior wall of the atrium; antrum indistinctly forked at its posterior end to form two rudimentary oviducts, but usually so distended with spermatozoa (thus acting as bursa seminalis) that no forking is evident; two large horn-shaped ovaries one on either side below the median frontal plane, extending from anterior fifth to middle of the body; two extensively branched and anastomosed vitellaria; ovaries and vitellaria not joined but closely apposed at their proximal ends where they meet the rudimentary oviducts; testes paired, situated one on each side just laterally and posteriorly to the pharynx; vasa deferentia short, arising from the inner posterior margins of the testes and opening into the seminal vesicle on its anterior surface; bursa seminalis lacking; viviparous, the young developing, usually in pairs, inside capsules of which as many as forty may be present at once in the mesenchyme of the mother; young break out of capsules and swim about in parent until they emerge through body wall or intestine; rolled up capsule shells remain in the mesenchyme.

Paul Hallez has given ('09) a detailed account of his observations on Paravortex cardii, a Rhabdocoele belonging to a genus closely related to Graffilla. The resemblance between Linton's and Hallez's species, as will be indicated below, is very striking.

The genus Paravortex was established by Wahl ('06) for a species of Turbellarian which is parasitic in the alimentary canal of Scrobicularia tenuis and S. piperata. According to Wahl it was discovered and inaccurately figured by Villot. Von Graff later called this parasite Macrostomum scrobiculariae, but Wahl asserted that it was worthy to be placed in a new genus. This he called Paravortex. He also found von Graff's Provortex tellinae to be identical with it.

Wahl ('06) briefly described the new genus Paravortex in the following words:

"Dalyelliide mit am Vorderende des Körpers gelegenem Pharynx, paarigen Keimstöcken, verzweiten Dotterstöcken, rundlichen Hoden und ventral vor der Körpermitte gelegener Geschlechtsöffnung."

In the same paper Wahl characterizes the genus Graffilla thus:

"Dalyelliide mit am Vorderende des Körpers gelegenem Pharynx, paarigen Keimstöcken von der Form gewunderen Bänder und davon getrenntren Dotterstöcken. Geschlechtsöffnung mittelständig, Hoden schlauchartig."

Concerning the presence or absence of a bursa seminalis Wahl says nothing, but his figures of the reproductive organs show very clearly that no such reservoir exists in Paravortex. We have already seen that this organ is characteristic of Graffilla.

According to Wahl, the genus Paravortex is distinguished from Graffilla, since its members have the genital pore situated in front of the middle of the body while in Graffilla this opening is at or behind the middle. The ovaries of Paravortex are club-shaped, while those of Graffilla are sinuous and band-shaped. The testes of Paravortex are nearly spherical; those of Graffilla appear bottle-shaped.

Von Graff gives ('08) a key to the Family Graffillidae, which is translated in part below:

- A. With two germo-vitellaria and two compact, unlobed testes.
    - 1. Pharynx well developed, germo-vitellaria not lobed.........Vejdovskya
    - 2. Pharynx small, germo-vitellaria lobed in the form of a hand..Paravortex
- B. With two ovaries and two vitellaria.
    - 1. Ovaries club-shaped, of normal size.
        - a. Vitellaria long and unbranched.
            - 1. Testes paired, genital pore on ventral surface....Paravortex
            - 2 Testis single, genital pore at or near the posterior end ....Collastoma
        - b. Vitellaria branched (Testes paired, genital pore near posterior end)...Umagilla
    - 2. Ovaries of different form, vitellaria branched or lobed.
        - a. Ovaries abnormally long cylindrical cords, vitellaria lobed in form of a hand, genital pore central.......................Graffilla
        - b. Ovaries and vitellaria hand-shaped, genital pore at the posterior end...Syndesmis

Thus it is seen that, according to von Graff, the genera Paravortex and Graffilla differ in that the former has two hand-shaped germo-vitellaria, i.e., the ovary and the branched vitellarium of each side unite at their proximal ends to form a single stalk which joins the oviduct. Graffilla, on the other hand, has two extremely long cylindrical ovaries which are not thus joined to the vitellaria; the latter, however, are branched.

Von Graff in his key did not refer to the receptaculum seminis, nor does he in the description of the genera dwell particularly upon this point. Nevertheless, while he fails to mention the organ in speaking of Paravortex, he notes that all the species belonging to the genus Graffilla are furnished with "eine mächtige Bursa seminalis" (receptaculum seminis.)

In order to classify Paravortex cardii Hallez ('09) presents the following table which brings out more clearly, as I think, the generic differences between Paravortex and Graffilla.

1.	A single ovary. Body more or less flattened	(2)
	Ovaries and vitellaria paired	(3)
2.	Ovary large, irregularly lobed. Genital pore posterior	Anaplodium
	Ovary small. Genital pore at the beginning of the last quarter of the body	Didymorchis
3.	Vitellaria unbranched	Collastoma
	Vitellaria branched or reticulated	(4)
4.	Genital pore posterior	(5)
	Genital pore ventral in the first half of the body	(6)
5.	Intestine lobed. A pharynx	Syndesmis
	Intestine straight. No pharynx in adult	Fecampia
6.	A bursa seminalis	Graffilla
	No bursa seminalis	Paravortex

From Hallez's key it appears that there is a disagreement as to whether the ovaries and vitellaria in Paravortex should be considered as one organ. Von Graff asserts that they are united, while Hallez considers them separate. From his description of P. cardii it is evident that Hallez is correct.

In comparing the genera Paravortex and Graffilla Hallex says, "Les genres Graffilla and Paravortex ont entre eux des affinités très étroites. La seule différence vraimente importante qui existe entre ces deux genres c'est l'absence chez Paravortex de la bours séminale qui se recontre dans toutes les espèces de Graffilla."

From a consideration of the several descriptions and keys quoted above one may summarise the distinction between the two genera Graffilla and Paravortex in the following manner:

Graffilla	Paravortex
1. Two extremely long cylindrical ovaries.	1. Two club-shaped ovaries.
2 Genital pore ventral, not anterior to the middle of the body.	2. Genital pore ventral, anterior to the middle of the body.
3. A large and distinct bursa seminalis (receptaculum seminis).	3. Bursa seminalis lacking.
4. No vestibule between pharynx and mouth.	4. Vestibule.

It is therefore obvious, since it agrees in all four of these characteristics, that Linton's species must be referred to the genus Paravortex. It now remains to determine wherein, if at all, the American species differs from the two found in Europe. Of Paravortex cardii Hallez gave a detailed description which, translated, is as follows:

"Vorticid provided with two ovaries, two reticulated and anastomosing vitellogenous glands and two spherical testes; bursa seminalis lacking. Genital pore ventral, situated close behind the pharynx, at the end of the first quarter of the body. Male copulatory organ muscular, without chitinous parts and bearing two lobes furnished with papillae. Pharynx doliiform. Mouth ventral, near the anterior end of the body. Body cylindrical, thinner at the anterior end, uniformly ciliated, white and with a slight yellowish tinge, transparent, without rhabdites, very contractile, ordinarily bent in an arc and turning about in one spot so as to describe a circle. Two black reniform eyes above the pharynx. Maximum length, 1 mm.; diameter 0.3–0.4 mm. Viviparous. Numerous soft-shelled capsules (up to forty) each enclosing one to four embryos and distributed through the connective tissue. Empty and rolled-up shells remaining in the body of the mother. Lives in the stomach of Cardium edule."

Linton's species and P. cardii are closely similar both in structure and habits. Both have essentially the same color and the same shape of body. Both have similar digestive, sensory and glandular organs; both give birth to living young which develop in capsules within the mother's body; both show the same peculiar movements when taken from their host and placed in sea water.

The two species differ, however, in that the American form attains twice the size of P. cardii; the genital pore is situated farther posteriorly and the ovaries are longer in the latter; an atrial canal in P. cardii leads from the dorsal part of the atrium backward to the antrum femininum, while in the American species there is no distinct canal but rather the antrum femininum extends backward from the middle of the posterior atrial surface and its opening into the latter is strongly constricted by a sphincter muscle; the openings of the shell glands in P. cardii are distributed along the entire ventral wall of the atrial canal and antrum femininum, while in Linton's species they all open at the anterior end of the antrum just back of the atrium; the vitello-oviducts of P. cardii are the longer. Linton's species lives as a commensal in the mantle cavity of the ribbed mussel, Modiolus demissus; P. cardii is parasitic in the stomach of Cardium edule.

Linton's species resembles Paravortex scrobicularia rather than P. cardii in the form of the ovaries, i.e., they are elongated in the first two and shorter in P. cardii. In form of antrum femininum and lack of an atrial canal Linton's species and P. scrobicularia are similar, but the latter, on the other hand, is similar to P. cardii rather than to the American form in that the atrium does not project dorsally beyond the opening of the antrum.

Since Linton's species obviously differs from the European forms, his specific name gemellipara is to be retained. Its applicability is owing to the fact that ninety-five per cent of the capsules contain two embryos.

2. Historical

While the anatomy and taxonomy of the Rhabdocoele Turbellaria have received considerable attention, only a few works have appeared dealing with their embryology. Hallez ('78) published the results of his observations upon the winter eggs of various Turbellaria. Since the young are enclosed within a hard-shelled capsule, the material was so difficult to study that he characterized these results as insufficient. In 1887 he did further work upon the fresh water Dendrocoeles.

In 1903 Caullery and Mesnil described the development of certain species of Fecampia, Rhabdocoeles parasitic in certain Crustacea.

One of the most complete and noteworthy contributions to the embryology of this group is that of Bresslau. In 1904 he published the results of his research, begun in 1898, upon the embryology of four species of the family Mesostomidae, namely

Mesostomum ehrenbergi, M. productum, M. lingua, Bothromesostomum personatum, and of one Alloiocoele, Plagiostomum girardi.

Another remarkable contribution to Rhabdocoele development is the section devoted to embryology by P. Hallez in his later work ('08), to which we have already referred, upon Paravortex cardii, a Rhabdocoele parasitic in the stomach and intestine of the mollusc Cardium edule. He therein reinterprets the significance of certain figures published by Caullery and Mesnil. As a result of the behavior of the ectoderm and entoderm cells to be described in this paper some of Hallez's figures in turn suggest an interpretation differing from his own. The condition at corresponding stages in the development of Paravortex cardii and P. gemellipara shows in many respects a striking similarity. In the formation of the ectoderm and the manner in which the vitellarial yolk enters the embryo, however, the process in P. gemellipara differs distinctly from that described for P. cardii. The ectoderm differentiation agrees essentially with that found by Bresslau in Mesostomum ehrenbergi. On the other hand, though the end result is the same, the manner in which the yolk is taken into the embryo of P. gemellipara presents, so far as the literature studied has revealed, marked variations from that described for any animal.

While Linton made no serious attempt to discover the origin of the twins in Paravortex gemellipara, he suggests that the twin condition might be due to a process of polyembryony. In order to settle this point J. T. Patterson in the summer of 1911 continued the study of the worm at the Woods Hole Laboratory. The results of his work appeared in 1912.

The purpose of the present paper is to describe the main features in the behavior and development of Paravortex gemellipara. Particular attention is to be paid to the processes of nutrition as taking place in all stages from the oocyte to the free-swimming worm, comparing them with homologous processes observed by investigators working on other forms.

It is a pleasure to express here my sincere appreciation of the kind assistance offered me in all stages of this research by Dr. Wesley R. Coe.

II. BIOLOGY, MATERIAL AND METHODS

Biology and material

Paravortex gemellipara was stated by Linton to be a commensal living on the gills of Modiolus demissus, but Patterson concluded that the worm lives primarily in the kidney, although he thinks it likely that many individuals escape and may then be found in the mantle cavity and on the gills. Patterson opened two dozen mussels, using care not to injure the tissues, When washed in water no parasites were found, but after tearing the kidneys of these same mussels apart, he secured thirty-eight specimens. Hallez ('09) found P. cardii in the stomach and intestine of Cardium edule. So, thinking that in attempting to dissect the kidney, Patterson might have cut the alimentary canal, I made several attempts between May and September to discover the worm in the stomach, intestine, kidney and liver.

After the adductor muscles alone had been carefully cut and the valves forced apart eight to sixteen mussels were vigorously washed in sea water. If more be opened at once the sediment renders the Turbellaria difficult to detect. The worms were allowed to settle to the bottom, and, the better to see the bottom of the dish, most of the water was slowly decanted. The vessel was then placed upon a dark surface in good light. In looking for the very young a hand lens was found useful. Next the stomachs of the same mussels were carefully slit open with dissecting scissors and the interior washed in another dish by means of jets from a pipette. Water was also forced through the intestine in the same manner. Finally, in a third vessel, the same mussels were again washed after tearing apart all the tissues, thus insuring the breaking open of the kidney, an organ extremely difficult to distinguish and dissect without disturbing other tissues.

It was found that two hundred thirty-seven mussels collected at various times during the year yielded with the first washing one hundred eleven specimens of Paravortex, with the second two and with the third six. In one instance thirty-seven worms were removed from eight mussels after the adductors had been cut, and only a single one when the viscera had been dissected. Considering the numerous crevices between the gills, visceral mass and mantle it seems very improbable that those worms which were not dislodged during the first washing were within the alimentary canal or kidney, but became freed from the mantle cavity in the two subsequent rinsings. The evidence points to the latter as being the usual abode of Paravortex gemellipara.

The number of worms obtained from different lots of mussels varies immensely. Patterson is of the opinion that there is a periodicity in the reproductive activity of this animal, but it is probable that the difficulty which he sometimes found in obtaining specimens was merely due to the irregularity of their occurrence. It has often been observed that one pailful of mussels may yield an abundance of worms, while another collected in a few hours and from the same mussel bed may produce very few. The conclusion is that only a portion of the mussels in the bed contain commensals. The success in obtaining specimens, therefore, depends upon the selection of the proper mussels.

Although usually no accurate count of the number of worms taken from given numbers of molluscs has been kept, a great variation is known to occur in their distribution. In the vicinity of New Haven Paravortex is abundant in certain mussel beds and almost entirely wanting in others. Any situation between high and low tide marks seems to be as favorable as another. Not always do those mussels which receive the most sunlight, even in winter, yield the greatest number of worms. In December, beds which were exposed to bleak winds sometimes gave better results than others which were fastened to rocks in sheltered coves with southern exposure.

Although, doubtless on account of increased metabolic activity and hence more prolific reproduction, Paravortex is more abundant in warm weather, still specimens may be obtained without difficulty at all seasons. I have collected them during every month of the year. Hallez asserts that P. cardii is as abundant in late autumn as in summer at Le Portel, nor does he find them less numerous in December and April. The greatest number of P. gemellipara obtained at New Haven was collected on November 9, 1912, when about 175 mussels yielded 310 worms. Only those easily detected with the naked eye were taken.

Linton states that he found this species negatively phototropic; that on leaving a dish containing specimens in strong light the worms were afterward found to have collected on the side opposite the source of light. My observations, on the other hand, point to a neutral reaction on the part of the older worms and a positive reaction of the younger. The adults are as often found on the light side of the dish as on the dark. Their observed tendency to secrete themselves beneath debris on the bottom of the dish was at first interpreted to signify that they were reacting negatively to light, but when it was ascertained that in a dish lacking such debris these older individuals seemed neutral it was concluded that thigmotropism was responsible for their hiding reactions. It has been noticed that an adult worm, upon coming in contact with a piece of mussel gill or other tissue, often crawls along in contact with it.

The young worms, on the other hand, are positively phototropic. If a number of them be placed in a watch glass and the latter then rapidly rotated so as to cause the collection of the worms at the center, as the water comes to rest they immediately swim vigorously toward the source of light. Only daylight was used. This experiment was made more striking by the introduction into the watch glass of several specimens of Aphanostoma. The latter, being negatively phototropic, as rapidly crawled to the darker side of the glass. Repeated trials were followed by the same reactions, the two groups of worms separating at the center and progressing in opposite directions.

There is a marked tendency on the part of the young worms to crawl up the side of the dish above the water line; death often resulted.

One can conceive that thigmotropism may be of value in causing the older worms to remain in the mussels. Linton suggested that negative reaction to light brought about this result, but as explained above it is extremely unlikely that the adults are thus negatively reactive to daylight. The marked positive reaction of the young specimens is probably responsible to some extent for their leaving the host within which they develop and so gives them an opportunity to enter other mussels. In the fulfillment of this end the negative geotropism of these young worms may also be of service; it would tend to cause a migration upward toward the source of light.

When removed from their hosts the healthy worms of medium size glide along the bottom of the dish at the rate of about one millimeter per second. Linton noted that the course in one direction was not long held, but often changed from one side to the other following a turning movement of the anterior end of the animal. Frequently these older worms halt and turn rapidly about for several revolutions in a circle whose diameter does not exceed the length of the individual (fig. 2). Hallez noted this circus movement as being extremely frequent in the case of Paravortex cardii.

The young worms move with greater rapidity for their size and in a much more direct course than the older invididuals. Furthermore they follow one direction often for several inches, only deviating in order to avoid obstacles. As already stated this course is consistently toward the light source. Rarely does a young worm perform the circus movement.

It is interesting to note in passing that Paravortex gemellipara like other Turbellaria observed by von Graff, is itself subject to parasitism. Frequently while studying living worms under the microscope numbers of an hypotrichous Infusorian are seen moving over them. One worm was so completely covered with them that, when the cover slip was pressed, these Infusoria became separated in such a way as to give the impression that the epithelium of the worm was peeling off. The hypotrichs, being ciliated only on one side, and closely approximating the epithelial cells in size, appeared strikingly similar to them.

Methods

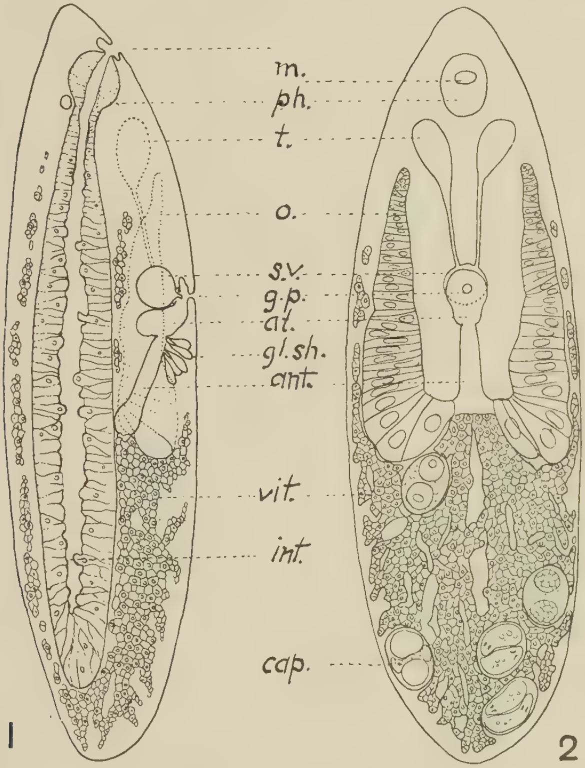
Observations made on the living worm slightly compressed beneath a cover slip enable one to make out the genital pore, ovaries, testes, seminal vesicle and vitellaria. But for the genital ducts this method proved unsatisfactory. In one instance a mass of spermatozoa was distinguished in the atrium of a specimen which lay with its ventral surface up.

The most reliable and complete data were obtained from sections of the worm. Of specimens killed in corrosive sublimate, Zenker's fluid and strong Flemming solution and subsequently stained with iron haematoxylin, those killed in Flemming solution gave the most satisfactory results, both as to preservation and stain. In most cases Orange G was used as a counter stain. Material fixed in corrosive-acetic and stained in Ehrlich's haematoxylin was excellent for the study of tissues in which the exact condition of the chromatin was of minor importance. Nuclei, as a whole, show much more plainly when treated by this method than when iron haematoxylin is used. Here again orange G was used as a counter stain. In connection with the study of sectioned material living embryos of various stages, removed by pressure from the mother, furnished checks on many points. A few useful total mounts were prepared.

III. ANATOMY OF THE ADULT REPRODUCTIVE ORGANS

The male organs

Like the related forms described by Bresslau, Hallez, von Jhering and von Böhmig, Paravortex is protandrous. In half-grown individuals the male organs are well developed. The testes lie one on either side of the body close behind the pharynx (fig. 3). A large seminal vesicle lies just above the genital pore which opens in the midline of the ventral surface about one-third the distance from the anterior end of the body. Two short vasa deferentia lead from the inner posterior margins of the testes to the seminal vesicle. A common atrium intervenes between the seminal vesicle and ventral body wall in such a way that the plug-like penis, situated in the small atrium masculinum at the lower end of the seminal vesicle, is directed through the common atrium toward the genital pore (text fig. 1). In no worm whose testes had become functional was the seminal vesicle found empty; usually it is distended with a mass of spermatozoa. All these organs are surrounded by a cellular wall.



Text fig. 1. Sagittal section of Paravortex gemellipara. × 167.

Text fig. 2 Optical frontal section from below. *ant.*, antrum; *at.*, atrium; *cap.*, capsules; *g.p.*, genital pore; *int.*, intestine; *m.*, mouth; *o*, ovary; *ph.*, pharynx; *s.v.*, seminal vesicle; *gl.sh.*, shell glands; *t*, testis; *vit.*, vitellarium. × 167.

In half-grown worms the male reproductive organs have reached maturity; after this period the testes with their ducts undergo partial degeneration. Patterson states that they are seldom found in large individuals, but I have seen them in every specimen examined.

Mitoses in the process of spermatogenesis are most readily observed in the younger worms; probably few spermatozoa are matured after the female organs begin to function. There is evidence, however, that this may happen. One series of sections of an old worm cut transversely shows a considerable number of apparently mature spermatozoa in the posterior dorsal portion of the testes and in the sperm ducts which can be traced for some distance backward toward the seminal vesicle.

The form of the spermatozoon is such as might, from its structure in nearly related animals, be expected. Von Graff ('02) figures and describes the spermatozoon of Graffilla buccinicola as 0.6 mm. in length with an oval head and a flagellum about twenty-five times as long. Von Jhering ('80) found in Graffilla muricicola spermatozoa some of which exhibited bifurcated heads with straight prongs. In others he was unable to observe this forked condition, and suggested that it might not be universal. Von Böhmig describes for the same species spermatozoa with heads thus bifurcated but with the prongs curved backward.

The spermatozoon of Paravortex gemellipara is composed of an unbifurcated head 0.01 mm. in length and an extremely slender cylindrical flagellum 0.10 mm. long (fig. 5). The head is slightly curved at the tip. The living spermatozoa are motionless while in the seminal vesicle, but when liberated in sea water they exhibit writhing movements. Evidently this behavior is not the normal mode of progression which takes place in the atrium and antrum, for no progress is made through the water; the spermatozoa merely twist about in one spot. Frequently they remain motionless in the water. Presumably the fluid inside the female passages ordinarily stimulates them to action.

The seminal vesicle in Paravortex gemellipara, as in the European species of Graffilla described by the German authors, functions not only as a reservoir but also as a 'ripening chamber.' Beside mature spermatozoa, many spermatids are usually found. Figure 48 was drawn from a living worm which had been compressed beneath a cover slip. In this seminal vesicle there were about twenty-five spermatids at a stage so young that the differentiation of the flagellum had but just begun. The nuclei could readily be seen in those cells which lay near the surface. Numerous spermatozoa were present as well. On another occasion a living animal while under observation on a slide became crushed. Among the mesenchyme cells which escaped from the body were many spermatids. Each cell had the form of a thick concavo-convex lens attached to the flagellum at such an angle as to give the spermatid the appearance of a ladle. These spermatids are always present in the seminal vesicles of worms whose ovaries have begun to set eggs free, but in the very young in which the testes have just commenced to function all the sperm cells are mature on leaving the testis.

Female reproductive organs

As the male organs degenerate those of the female apparatus undergo rapid development. By the time the animal is two-thirds grown the ovaries and vitellaria are prominent. The ovaries are situated one on either side of the body below the median horizontal plane. At the small anterior end of each ovary, which lies just posterior and slightly dorsal to the testis of the same side, numerous oogonia are crowded irregularly together. Cell membranes can be distinguished except between the first two rows of nuclei. In some specimens a terminal filament passes forward from the tip of the ovary and joins an indistinct mass of cells on the posterior ventral surface of the testis. As will be seen in the part of this paper which treats the development of the reproductive glands, the ovary and testis arise from a common mass of undifferentiated cells, and later usually become separated. Where in later stages this strand of cells remains between the two glands it is regarded as a bridge which has failed to break down.

Striking spireme figures are to be observed in the oogonia. A short distance back from the anterior end of the ovary, however, only cells with resting nuclei occur. These are primary oocytes. Beyond this point the flattened cells are packed closely together one behind another, as Patterson suggests, like a "rouleau of coins." The ovary increases greatly in diameter toward its posterior end. At about the mid-length of the animal it turns inward to meet the oviduct (figs. 3, 4; text fig. 2).

The enormous paired vitellaria discharge their contents into the same ducts as the ovaries. Each vitellarium sends branches anteriorly and posteriorly. At the height of their development the numerous anastomosed branches completely surround the intestine, occupying practically the whole region between it and the body wall (fig. 3). The anterior lobes lie above the ovaries and intestine; in extreme cases a few branches grow forward to a point above the brain. Single rows of yolk cells constitute the ultimate lobes. Indeed, individual cells frequently lie isolated in the parenchyma.

So closely do the vitellaria press upon the ovaries at the posterior ends of the latter and for some distance along their dorsal surfaces that the two organs occasionally appear to be joined into a single germo-vitellarium on each side of the body. As a matter of fact, however, if they join at all it is only at the point where they meet the oviduct—or better, the 'vitello-oviduct.' The stalks of the two vitellaria, as seen in figure 4, are separated only by a thin partition of mesenchyme. No cellular wall has been observed between the ends of the oviducts and the vitellaria. As shown in this figure, yolk cells often appear in the upper end of the antrum, indicating that they are likely to be liberated by any slight pressure in this region.

Posterior to the seminal vesicle a dorsal diverticulum of the atrium commune is considered by Patterson to be the degenerate seminal receptacle, although he found no spermatozoa in the sac. In several of our preparations it contains a mass of spermatozoa (fig. 9) which so distend it that the top is on a level with that of the seminal vesicle. This organ is rather to be regarded as a bursa copulatrix than as a receptaculum seminis; the spermatozoa, during copulation, are first received in this dorsal pouch of the atrium commune and afterwards pass into the antrum femininum. The atrium is lined internally with a high columnar epithelium, externally by a single layer of flat cells (fig. 51). A comparison of text figure 1 with Hallez's brings out the difference between the two species as regards the shape of the atrium, atrial canal and antrum femininum. In P. cardii the atrium rises only a very little above the opening into the atrial canal, while in P. gemellipara the antrum opens into the atrium at the middle of its posterior surface. It was stated in the introduction that the lack of a bursa seminalis distinguishes the genus Paravortex from Graffilla.

The delicate-walled antrum femininum (text figs. 1, 2) extends from the atrium commune posteriorly and dorsally to a point beneath the intestine. Strong sphincter muscle fibers constrict the opening between antrum and atrium. So delicate are the walls that only occasionally can the cells be made out. Figure 4, referred to above, was drawn from a horizontally sectioned animal which showed the walls of the antrum exceptionally well. The section chosen included only the upper end of the antrum; the rest of the structure must be conceived as lying below the plane of the paper. No marked bifurcation is evident here on account of a distention with yolk cells; the ends of the vitellaria border the whole posterior surface of the antrum, while the terminal egg in each ovary presses inward against its outer angle. No uterus homologous to that found in many Turbellaria is present in Paravortex. In this regard the following extract from von Böhmig's contribution to Bronn's Klassen und Ordnungen des Thier-Reichs is of interest:

"Bei allen diesen, dient, wie u. a. schon Böhmig für Graffilla muricicola und Vedjovsky für Phaenocora hervorgehoben haben, das Atrium commune oder Antrum femininum als Uterus . . . zugeschrieben werden."

Beneath the antrum and posterior to it are groups of single-celled shell glands. Each contains a prominent nucleus and a considerable amount of granular cytoplasm (figs. 3, 9). Although it is difficult to determine the exact point at which these glands are attached to the reproductive tract, they all converge toward the lower end of the antrum near its union with the atrium. As noted above, this character helps to distinguish

P. gemellipara from P. cardii in which the shell glands open along the entire central surface of the antrum.

Although no one has recorded observations of Graffillidae in the act of copulation, it is inferred that pairing occurs. During the process, as described for other Turbellaria, masses of spermatozoa from the seminal vesicle of one animal are introduced by means of the penis into the atrium of its mate. There may be a mutual exchange, but certain observations lead to the belief that the younger specimens in which the male organs are at the height of their development fertilize the older ones having the functional female apparatus, while older ones do not fertilize the younger.

In this regard it is to be noted that during early life the animals have widely distended seminal vesicles; in the old worms this organ contains only small masses of spermatozoa. Moreover the younger specimens in which the ovaries are not functional have not been observed to contain sperm in the atrium and antrum.

Embryos

In a worm which is rapidly reproducing one finds distributed through the parenchyma capsules (fig. 1) containing embryos in various stages of development, from the newly-fertilized egg to ciliated young, actively moving about in their cramped quarters. During early development the two embryos of each capsule are usually separated by a considerable thickness of yolk, but may in some cases lie so close together that the blastomeres of one touch those of its mate. The capsular membrane, heavy and well defined while the embryos are small, becomes gradually thinner on account of water absorption and consequent stretching until, by the time the young develop cilia, it is extremely delicate and apt to be ruptured by vigorous pressure of the surrounding organs. When fully enough developed the young break forth from their capsules, swim freely about in the mesenchyme of the mother for a time, and ultimately, as her body undergoes degeneration, slip through the body-wall to the outside. Frequently one is found in the mother's intestine.

IV. DEVELOPMENT

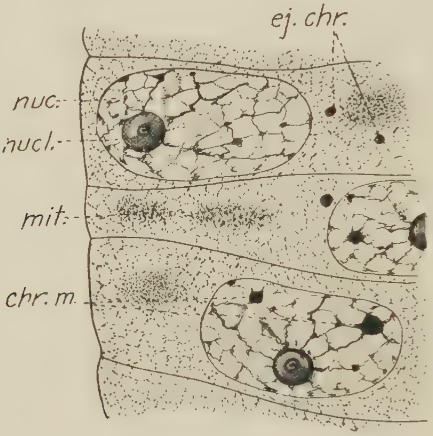
1. *Growth of the eggs and consideration of the yolk-nucleus (mitochondrial mass)*

Throughout the entire length of the ovary (text fig. 2) from the point where the oogonia are transformed into oocytes there is a gradual increase in the size of the latter. The nucelus measures at the beginning 0.01 mm.; at the time it leaves the ovary its diameter is about 0.025 mm. During all this growth period the nucleus is in a resting condition, showing a fine chromatin reticulum and prominent nucleolus. The latter is at first apparently a sphere whose periphery retains more stain than the center. Often one side of the nucleolus is much darker than the other, so that the pale interior is eccentric. When the cell has passed half the length of the ovary a dark spherule with a light interior appears in the center of the nucleolus (figs. 7, 8), and remains until the cell leaves the ovary.

The term 'resting nucleus' is to be used here only in so far as it applies to the nucleus as a whole; i.e., it retains its spherical or oval form and its limiting membrane. There is observable, however, a certain activity of the chromatin. While the newly formed oocyte nucleus contains a heavy chromatin reticulum whose strands are quite uniform in thickness, the latter soon became markedly thinner. This is due to the accumulation of a part of the chromatin in one to four masses usually near the nuclear membrane (text fig. 3). These are at first irregular, but soon become rounded so as to resemble the nuleolus in form. Rarely do they attain the size of the latter, however, and never contain a lighter interior; an intense blue-black iron-haematoxylin stain is characteristic of these fragments.

It is first at this stage that one often finds one or more black bodies of perfectly spherical outline lying in the cytoplasm outside the nucleus. Their appearance is such as to suggest their identification with the chromatin bodies just described as arising in the chromatin reticulum. If now the nuclei of the cells in whose cytoplasm these black spheres lie be examined, it is found either that they contain only a nucleolus and a fine chromatin reticulum, or that fewer black masses are present than in the oocytes which are slightly younger. It seems probable then that the oocyte nuclei eliminate a part of their chromatin in the form of spherical fragments which come to lie in the cytoplasm.

Of considerable interest in relation to the nourishment of the embryo is a body always found present in the oocyte after it has commenced its growth. This is the 'Dotterkern' or 'yolk-nucleus.' A light brown color in iron-haematoxylin-eosin preparations brings the yolk-nucleus into contrast with the cytoplasm in which it is imbedded (figs. 4, 6, 7). At a magnification of two hundred fifty its appearance is that of a homogeneous gelatinous mass, sometimes existing as a single compact body, but often as flocculent material in one or more smaller masses of different sizes. The substance consists ultimately (fig. 7) of a multitude of granules which are so extremely fine and so closely grouped that a high magnification is necessary in order to distinguish them as such.



Text fig. 3 Portion of a frontal section of the ovary to show masses of chromatin before and after ejection from the nucleus. *chr.m.*, chromatin mass; *ej.chr.*, ejected chromatin; *nuc.*, nucleus; *nucl.*, nucleolus; *mit.*, mitochondrial mass. × 1016.

Since the term 'yolk-nucleus' has been applied by different authors to several different cell structures in many groups of animals, it seems advisable to pause here, and briefly to describe these various types. The name 'Dotterkern' was first given by Carus in 1850 to a body which had been described by von Wittich as lying in the cytoplasm of immature spider eggs (Lycosa, Tegenaria and Thomisus). Since then observers have reported the presence of a yolk-nucleus in the eggs of echinoderms, molluscs, worms (Trematodes), crustaceans, myriapods, insects, fishes, amphibians, birds and mammals, including man.

As explained in Korschelt and Heider's Textbook of Invertebrate Embryology, the bodies described for the various animals as yolk-nuclei may be classified in three groups, (*a*) true 'Dotterkerne' which are supposedly concerned with the deposition of yolk in the egg, (*b*) bodies more or less similar to yolk-nuclei in appearance, and (*c*) structures which are to be identified as attraction spheres.

Calkins in his preliminary notice published in 1895 on the origin and changes of the yolk-nucleus in the eggs of the earthworm, Lumbricus terrestris, points out that "the work of various observers may be divided into two classes according as the yolk-nucleus is conceived to be of cytoplasmic or nuclear origin."

As examples of the first class Calkins cited, among others, the work of Lubbock ('61) who regarded the yolk-nucleus as a thickening of the plasm forming the vitellus, of Sabatier('83) who asserted that in the spider the yolk-nucleus arises in the cytoplasm near the nucleus and wanders to the periphery where it degenerates; of Hall ('90) whose observations on the chick led him to the conclusion that the yolk-nucleus has its origin in a mass of granules near the germinal vesicle to which it may send out prolongations; and the work of Monticelli ('92) who claimed that in the Trematodes there is no connection between germinal vesicle and yolk -nucleus, but that the latter arises as a protoplasmic differentiation of the cytoplasm. Jordan ('93) came to a similar conclusion in regard to the yolk-nucleus of the newt.

Of investigators who considered the yolk-nucleus to be derived from the nucleus Calkins mentioned Shäfer ('83) who observed in the fowl's egg that the yolk-nucleus was connected with the germinal vesicle by numerous fine striations; Balbiani ('83) who claimed that in the Geophilidae it originates by direct transformation of the parts of the nucleolus of the germinal vesicle. Leydig ('88) saw the nucleolus in Triton become amoeboid and wander out into the cytoplasm where it finally broke up into granules around the periphery of the yolk. Finally he cites Henneguy ('93) and Balbiani ('93)who both found that in various eggs the yolk-nucleus originates from the nucleolus.

As a result of Calkins' own observations ('95) on the eggs of Lumbricus he concluded that the yolk-nucleus is of nuclear origin. He found it lying on the margin of the nucleus in direct connection at several points with the chromatin; the nucleolus takes no part in its formation. After migrating into the cytoplasm he conceived the granules of which it is composed as separating and forming the yolk plates after undergoing a change in chemical composition.

Wilson ('96), in "The Cell in Development and Heredity" speaks broadly of the yolk-nucleus, conveying the impression that already the body was beginning to be looked upon as not having, perhaps, so much importance as a functional cell organ as had previously been attributed to it. He says in part:

> Calkins' observations taken in connection with those of Balbiani, Van Bambeke, and other earlier workers give. however, strong evidence, as I believe, that the 'yolk-nucleus' of Lumbricus is derived, if not from the nucleus, at any rate from a substance nearly related with chromatin, which is afterward converted into cytoplasmic substance. It is certain, in this case, that the appearance of the yolk-nucleus is coincident with a rapid growth of the cytoplasm; but we cannot suppose that the latter grows entirely at the expense of the yolk-nucleus. More probably the yolk-nucleus supplies certain materials necessary to constructive metabolism, and it is not impossible that these may be ferments. We may perhaps interpret in the same manner the elimination of separate nuclear elements, (i.e., not forming a definite yolk-nucleus) as described by Van Bambeke, Mertens, v. Erlanger and many earlier writers.

> The meaning of the yolk-nuclei of purely cytoplasmc origin is very obscure, and we have at present really no ground for assigning to them any particular function. It can only be said that their appearance coincides in time approximately with the period of greater constructive activity in the cytoplasm but there is no evidence of their direct participation in the yolk-formation, and we do not know whether they are active constructive physiological centers, or merely stores of reserve substances or degeneration products.

Crampton in 1899 published an account of the 'yolk-matrix' of an Ascidian egg (Molgula). He first noted its appearance in the living primary oocyte as "one or more small masses of highly refractive granules" lying in the cytoplasm at one side of the nucleus. Later, the granules become more numerous and are closely packed together into a cap-shaped mass which partly encloses the nucleus. Various chemical tests led him to the belief "that the yolk-matrix is an albuminous body of the cytoplasm, but perfectly agrees with the albuminous constituents of the nucleus," and that on this ground the yolk-nucleus is either directly derived from the nucleus or is formed under its immediate influence.

After a time the granules constituting the yolk-matrix separate, according to Crampton's observation, and spread throughout the cytoplasm in whose interalveolar substance they came to lie. Finally the granules enlarge to form the deutoplasm-spheres.

In the light of more recent research it would appear that these yolk-nucleus granules are mitochondria, and that Crampton's second alternative as to their origin is the correct one i.e., that they are formed under the influence of the nucleus, rather than directly from nuclear material.

One of the most prominent types of yolk-nucleus was described in 1898 by van Bambeke for the oocyte of the spider, Pholcus phalangioides. First appearing as a small group of granules near the nucleus, it develops into a definitely outlined, more or less cresentic mass lying in a clear portion of the cytoplasm. Increasing immensely in size the body grows around the nucleus, sometimes adhering to it for a time, but finally reaching the height of its development in the form of a cup within whose hollow the greater part of the cell body lies. In other words, the yolk-nucleus is peripheral. In some sections it appears as a crescent reaching more than half way round the cell; in other planes which are at nearly right angles to the last a ring-like band is the picture presented by the yolk-nucleus.

From this point on Van Bambeke noted a disintegration of the 'corps-vitellin.' The essential difference in the fate of the granules into which this body fragments from that ascribed to them by earlier investigators of other forms, lies in their transformation into fat globules which are subsequently 're-sorbés etassimilés par le protoplasme ovulaire." In view of this van Bambeke suggests that it is on account of the resorption that the protoplasm is able to elaborate the deutoplasmic elements. It will be remembered that Calkins and Crampton recognized a direct transformation of yolk-nucleus granules into yolk spheres.

According to von Adelung in the Zoologisches Centralblat Samassa and Groben described for the parthenogenetic egg of the Daphnid, Moina rectirostris, an intensely staining body of characteristic form, which they called the polar body. Weissman and Ishkawa, however, observed the formation in the living egg of the true polar body. Finally Lepechkine in 1900 determined that the structure observed by Samassa and Groben is a true yolk-nucleus, and characterized it as follows

> Er ist dem Ei von dessen Ablage an bis zum 32-zelligen Stadium eigentümlich, wird durch Kernfärbemittel tingiert, gehört von Anfang an einem bestimmten, am animalen Pol gelegenen Blastomer an, ist von bestimmter Gestalt, gleichmässiger Konsistenz und enthält Vakuolen, löst sich beim Zerfall gleichsam in feinste Körnchen auf, wobei diese, dem Kern sich nähernd, denselben umgeben; der Dotterkern steht in Verbindung mit dem Kerncentrosom, welches seinen Zerfall herbeiführt; nach dem Zerfall des Dotterkerns treten in dem entsprechenden Blastomer Fettröpfchen auf, welche auf lange hinaus einem Bestandteil dieses Blastomers sowie seiner Tochterzellen ausmachen; aus dem den Dotterkern enthaltenden Blastomer enstehen die Genitalzellen des Keims; endlich giebt der Dotterkern in den ersten Furchungsstadien ein Plasmaklümpchen ab, welches zwischen die Eioberfläche und die Dottermembran zu liegen kommt.
>
> Was die Bedeutung des Dotterkerns betrifft, so ist der Verf. (mit van Bambeke, Wilson und Häcker) der Ansicht, dass derselbe eine

Rolle im Stoffwechsel spielt, und die Entwickelung und das Wachstum entweder das ganzen Eis oder eines bestimmten Blastomers begünstig.

From the foregoing review it is evident, first, that several different cell structures have been designated as yolk-nuclei, and second, that diverse views have been held as to the origin, function, if any, and the fate of the 'Dotterkern.' In the majority of instances where the latter was traced its disappearance as a concrete cell organ occurred in the oocyte. A part of the yolk-nucleus observed by Balbiani in Tegenaria, however, retained its individuality throughout cleavage and finally became located among the yolk-spheres in the abdomen of the young spider.

Since Lepechkine's work of 1900 a vast amount of literature has appeared dealing with mitochondria. It is apparently to this class of elements that the true yolk-nuclei described by him and earlier workers belong. Therefore it seems justifiable to replace the name yolk-nucleus with one which is more descriptive of its true nature. We propose to use in this paper the term 'mitochondrial mass.'

The cytoplasm or ooplasm of the oogonia is, in well preserved preparations, so finely granular that it resembles an opaque fluid. With the iron-haematoxylin method a light bluish-gray color is characteristic. If the sections be washed too long in the iron alum after their removal from the stain, and subsequently counter stained with orange G, the latter imparts to the cytoplasm of the oogonia and oocytes a yellowish brown tint. In such mounts the mitochondrial mass, which also takes the orange stain, is detected with difficulty. Those preparations, however, which retain a sufficient amount of the blue haematoxylin show the mass even in its earliest stages. Figure 6 was drawn from such an ovary. A small group of brownish granules in the third oocyte which shows a membrane on the right hand side of the ovary is the first indication of the mitochondrial mass. A study of the nuclei in the younger cells does not lead to the conclusion that the granules are of nuclear origin. They are rather to be regarded as bodies which have arisen in the cytoplasm under the influence of nuclear energy.

The question as to whether any function is to be assigned to the mitochondrial mass in Paravortex can be more intelligently considered after the conditions under which it exists as long as it remains visible have been fully described.

At a time approximately coincident with the appearance of the mitochondrial mass the cytoplasm of the oocyte becomes less dense, while its general color changes from the bluish gray of the oogonium to a more neutral tint bordering on the brown. So far as can be observed with the highest magnification obtainable this change in color is due not to an intercalation of yellowish particles but to a change in the staining reaction of the cytoplasm as a whole. The simultaneous increase in the amount of the latter is not caused by deposition of distinct deutoplasm spheres. At any rate there is no addition of particles which stain differently from the cytoplasm. Neither is growth the result of vacuolization; the mature oocytes in well preserved material never contain vacuoles. The only change observable, in so far as the consistency of the cytoplasm is concerned, is a tendency of the minute granules to become grouped in slightly larger units of irregular form. The appearance presented to the eye is that of a multitude of small flakes and flocculent bars between which intervene irregular colorless regions which in size closely approach that of the stained particles (fig. 7). These spaces become somewhat more conspicuous as the oocyte reaches maturity. It has been impossible to detect in them the 'mitosomes' which Wilson and others have described as lying in the protoplasmic trabeculae between the alveoli of the echinoderm egg.

Although, as pointed out above, no conspicuous spheres are to be found in the cytoplasm of Paravortex, it is apparent that the particles which are added must be of the nature of deutoplasm. Their distribution is remarkably homogeneous; in other words, the eggs are homolecithal or alecithal.

Nourishment enters the ovarian cells by osmosis from the parenchyma surrounding them, not, as Patterson states, in the form of particles ingested from the vitellaria which at some points come into close proximity with the ovary. The vitelline cells contain flakes and globules of a substance which, by the haematoxylin-eosin method, are stained an orange brown, and are in size and density much more conspicuous than the particles suspended in the cytoplasm of the oocyte.

During the growth of the latter the mitochondrial mass has assumed conspicuous proportions. This relative increase in bulk is well illustrated in figure 6, which indicates as well that the substance may be accumulated in a single mass, or irregularly distributed throughout the cytoplasm. One might easily imagine, on account of the uneven edges of the masses from which lines of granules are often seen extending out into the cytoplasm, that the mitochondria are being directly transformed into the deutoplasm particles. Van Bamkebe ('98) points out that it is well to guard against such a conclusion, since he finds in the oocytes of Pholcus that the 'yolk-nucleus' granules first become transformed to fatty drops which are reabsorbed by the protoplasm, and suggests that possibly the latter then manufactures from this nutritive material the true deutoplasm globules. For the present, judgment in this matter of the function of the mitochondrial mass is suspended. It is to be noted that the body increases consistently in bulk while the cell is still in the ovary.

At the time of encapsulation the oocyte has attained a size about four times that which characterized its earliest appearance. The average measurements are approximately 0.112 mm. in width and 0.023 mm. in length, the cells being strongly flattened perpendicularly to the long axis of the ovary.

2. Formation of the capsule and origin of the twins

As to the manner in which the older eggs are nourished, Patterson asserts, "that the ova are at their upper margins absorbing yolk from the glands" (the vitellaria). This altogether erroneous interpretation will be considered below with another concerning the origin of the twins. Again Patterson states that,

> In consequence of this rapid growth certain retrogressive changes involving the cell membranes separating contiguous ova frequently

> make their appearance. As a result two or even more nuclei may come to lie within one common yolk mass, which occupies the extreme tip of the ovary. In other words, a syncytium is formed here. In the vast majority of cases only two ova are involved so that the usual picture displayed in this region represents a binuclated yolk mass.

Patterson was evidently misled, both as to the relation of the ova to the cells of the vitellaria, and their relation to each other, by the phenomenon of oblique sections across thin membranes. Hardly a better example of this condition could be found than a longitudinal section of the Paravortex ovary, particularly of its posterior end where it turns inward to meet the oviduct. At this angle several of the ova are sure to be cut with varying degrees of obliquity, rendering the egg membranes correspondingly indistinct. The membrane between the last two ova included is the most likely to be cut at the sharpest angle, and hence the most difficult to observe.

Since all the eggs in the ovary are flattened nearly perpendicularly to the horizontal plane, a frontal section of the organ is all that is necessary to prove that very distinct membranes do exist between all ova, particularly at the posterior end of the ovary (fig. 8). A vertical section cut obliquely through the posterior angle of the ovary brings out the same fact.

Patterson's figure, introduced as evidence in favor of his syncytium theory, indicates very clearly to us that the ovary curved inward at the right. The egg having the larger nucleus was pressed into its neighbor containing the smaller nucleus. Since the plane of the section was more tangential to the first than to the second the former appears to be contained within the boundaries of the latter. The membrane between the two appears indistinct, not because it is undergoing degeneration, as Patterson would interpret the condition, but on account of the obliquity of the plane at which it is cut. Many of my preparations present an identical appearance.

In a similar manner I would account for the faintness of the boundaries between ova and vitelline cells in the same figure. Sections through the posterior end of the ovary and the overlying vitellaria are not usually perpendicular at all points to the membranes of the eggs and of the vitelline cells, owing to irregularities in the contour of the ovary. Wherever the section is not thus perpendicular it is oblique, and hence the membrane is inconspicuous.

The masses of granules which Patterson interprets as streams of yolk entering the ova can be found in eggs which are not in contact with the vitellaria. In my own preparations I have considered these darker lines of granules as portions of the cytoplasm which retained more stain than that the rest of the cell.

It is evident, then, that Patterson was misled, both in regard to the relation of the contiguous ova in the posterior end of the ovary, and the relation of the ova to the vitellaria. Therefore I cannot agree with him, either that the vitelline material is pumped directly into the ova, nor that the breaking down of a membrane between two ova is part of a process by which two or more nuclei are enclosed in one capsule. No syncytium is found here. I am not content with the qualification which Patterson introduces as follows:

"However not in all cases do the two contiguous ova lose their intervening membranes, but some become completely surrounded by vitelline cells, which through a process of disintegration, form the yolk mass of the definitive capsule." The egg membranes are never so lost. It can be shown that in order reasonably to account for the multiplicity of embryos in capsules of Paravortex gemellipara it is unnecessary to resort to such an extraordinary mechanism as a syncytium in the ovary.

As to the manner in which two embryos come to lie in one capsule two possible hypotheses remain; first, that polyembryony occurs in P. gemellipara, or second, that two or more ova are liberated, and, together with yolk cells from the vitellaria, become simultaneously enclosed within one capsular membrane. That the first hypothesis is false there is no room for doubt. Capsules have often been observed in which both ova are undergoing maturation, a state which precludes the possibility that a single fertilized egg was enclosed in a capsule and subsequently divided into two cells, each capsule developing into an embryo.

There remains the hypothesis that any capsule which contains a certain number of embryos originally had deposited in it the same number of eggs. A priori it is to be expected that such a process occurs here, for both von Jhering ('80) and von Böhmig ('86) observed in the uteri of the oviparous parasites Graffilla tethydicola and G. muricicola respectively capsules containing usually two ova; Böhmig states that each ovary contributes one egg. The same mechanism is probably involved in the formation of the capsules of the oviparous specimens mentioned above and in the viviparous Paravortex gemillipara. In the former two species the capsules pass out of the animal through the atrium and genital pore, while in the case of P. gemellipara they never enter the atrium, but break out of the antrum into the mesenchyme.

Finally Hallez ('09) observed the formation of the capsule in Paravortex. To quote:

> Chez Paravortex cardii, comme dans l'espèce précédente, il n'existe ni utérus, ni cavité d'aucune sorte au point de jonction du lécithogène, de l'ovaire et de l'oviducte.
>
> Quand le cocon commence à se former, il se produit un afflux de cellules vitelline autour des ovules mûrs qui ne sont pas encore complètement détachés de l'ovaire, afflux qui distend la membrane propre de l'ovaire et refoule le tissue conjonctif latéralment, c'est-à-dire du côté où la résistance est moindre. Il se forme ainsi une hernie qui s'accroît davantage à mesure que le nombre des cellules lécithofères augmente autour des ovules mûrs . . . .
>
> On observe à ce moment des spermatozoïdes dans la partie de l'oviducte voisine de l'ovaire . . . , tandisque l'atrium femelle et une partie de l'oviducte contient un cylindre d'une substance amorphe, transparente, coagulée par les réactifs . . . . Cette substance, qui ne peut être que la produit de sécrétion des glandes coquillières, adhère par places à la paroi de l'atrium comme si elle était glutineuse.

Hallez finds that from two to four ova are thus inclosed in a capsule. Since the ovaries of P. cardii consist at their posterior ends of several eggs, there is greater likelihood that more than one at a time will be freed than there is in the case of P. gemellipara. As a matter of fact Hallez finds a larger number of capsules containing three or four embryos than is met with in the latter species.

a. The process in Paravortex gemellipara. The general plan and appearance of the ovaries, vitellaria and antrum of P. gemellipara supports the hypothesis that a similar process occurs here. As described in the introduction, the antrum femininum bifurcates at its distal end, sending a thin-walled branch, the oviduct, to meet the ends of the ovaries and vitellaria of each side.

Examination of the posterior end of either ovary in animals which have reached the egg-producing stage usually shows that the last, and hence the ripest, egg is being pressed inward toward the antrum by the ova behind it. Posteriorly the compact vitelline cells crowd the egg in a forward direction so that it appears, in a frontal section, as in figure 4. Here the terminal egg of each ovary is being subjected to the same conditions. The one on the right seems just in the act of being forced into the oviduct. Such is the usual picture, an egg from each ovary on the verge of entering the oviduct. Only a slightly greater pressure is required to complete the process. Suppose the worm were now to contract its body in this general region; the probable result would be that both ova would enter the antrum.

Most conclusive visual evidence is to be gained from a series of sections of a specimen which had been killed in the most critical stage, where two eggs had just been set free and were being surrounded with the yolk cells from the vitellaria. The sections are slightly oblique between a vertical longitudinal and a frontal plane, somewhat nearer the latter.

Figure 9 drawn from the specimen under consideration, shows what is taking place. Apparently the right ovary, part of which is included in the section, was distorted and crowded inward by the contraction of the animal in the fixing fluid. Otherwise it would have been impossible to have cut a section, which would include, as this does, seminal vesicle, antrum and ovary. Aside from the retention of a deeper stain these two ova are in the same condition as the eggs at the end of the ovary. The nuclei of both are resting, and their chromatin and nucleoli are typical of ripe ova in which maturation has not yet begun. Owing to a relief of pressure both eggs are more nearly spherical than those in the ovary.

The yolk cells are arranged about these eggs in a loose envelope on all sides except that toward the ovary. Between the yolk cells and the eggs numerous spermatozoa appear. The latter can be traced throughout the length of the antrum and into the atrium where they form a compact mass.

This specimen leaves no doubt that two normal ova have been freed into a temporary cavity formed at the thin-walled distal end of the oviduct and partly bounded by the ovary, vitellarium and mesenchyme, and are being surrounded with a number of vitelline cells, between which numerous spermatozoa were actively moving at the moment of fixation. No proof is at hand that one of the eggs came from the right ovary and the other from the left, nor that both came from the right; either case is possible. Their presence near the end of one ovary may signify that both ova were derived from the latter. On the other hand, the possibility is not excluded that one egg came from the other ovary, and by pressure of the tissues was quickly shifted to its present position. In favor of this view it is to be observed in this series of sections that, while the right hand ovum is still in contact with the outer corner of the last egg in the ovary, its mate is entirely free, and has assumed a more nearly spherical shape—two characteristics which an egg would probably possess had it come from the other side of the body.

That one ovary may contribute two ova to the formation of some capsules, is clear when it is known that occasionally three embryos are found in a capsule. As stated in the introduction, two is the normal number, but Linton ('10) and Patterson ('12) both observed capsules with one and three embryos. My material shows further examples of both; and in addition one capsule containing four. Thus it is possible that the two eggs deposited in any capsule may have had a common source, or may have come from opposite sides of the body. The weight of the evidence favors the latter as being of most common occurrence in Paravortex gemellipara.

In figure 9 are shown several masses of a homogeneous substance which is altogether unlike anything observed in other preparations. This latter fact, together with the arrangement of the masses—more apparent in other sections of the same capsule—in a half circle about the yolk cells which surround the two ova, suggests that the substance is the fluid secretion from the shell glands in the process of being laid down in the formation of the capsular wall. A similar condition was observed by Hallez ('09) for Paravortex cardii.

As the above quotation from his paper points out, Hallez asserts that there is no uterus in Paravortex cardii nor a cavity of any sort at the point of junction between vitellarium, ovary and oviduct. Still he figures a direct continuation of the lumen of the oviduct into the cavity which is receiving a mass of yolk cells. It is true that P. gemellipara possesses no permanent, distinctly limited uterus by whose contractions the yolk cells and ova are molded into a spherical mass to which by accretion the capsular fluid is applied. Nevertheless it is apparent that in P. cardii and P. gemellipara the process is essentially the same as in oviparous worms, differing only because the encapsulation cavity is bounded partly by the ruptured and distended end of the antrum femininum and partly by the vitellaria, mesenchyme and ovary. The cavity is indefinite and probably never exactly the same for successive capsules, but there is, in spite of the delicateness of the tissues, sufficient resistance to round off the capsular contents and allow of the laying down of the shell. Thus the process is essentially in agreement with that found by other investigators in related species.

b. Description of the newly formed capsule. The appearance of a capsule which has just passed from the reproductive organs into the body parenchyma is clearly shown in figures 10 and 11. It lay close outside the uterus, slightly posterior and ventral to the point at which the ovary turned inward to join the vitellaria. It should first be made clear that, whereas living capsules due to their turgidity are smooth in outline, preserved material shows capsules somewhat shrunken and crowded out of shape by neighboring tissues.

The shell membrane is clearly visible, on one side closely opposed to the yolk, while on the other a shrinkage space intervenes. This capsule contained but one egg. That illustrated in text figure 4 shows the two ova at once. Only exceptionally do eggs lie so near the surface as indicated in figures 10 and 11; usually they are until late cleavage entirely surrounded by yolk. In figure 11 the plane passed through portions of eleven yolk cells, of which there were, as determined by a count of the visible nuclei, thirty-six originally enclosed in the capsule. Hallez found from ninety to one hundred eighty-seven in various capsules of P. cardii. As may be seen by referring to figure 10 of another section through the same capsule several cells have already lost their membranes; only the degenerating nuclei remain to indicate the number of cells.

c. *Behavior of the vitelline cells.* Up to this point, with the exception of the yolk nucleus above described, the structure and behavior of the reproductive organs and their products are remarkably similar in P. cardii and P. gemellipara. From here on, however, the latter presents characteristic differences as to the fate of the vitellarial yolk-cell nuclei, absorption of the yolk and the formation of the ectoderm.

The ectolecithal yolk cells in the P. cardii capsule, according to Hallez, soon lose their membranes, those near the center retaining them the longest. About one-half of their nuclei, it is to be noted, degenerate, while the remainder come to lie in the portion of the yolk which stains like cytoplasm. From this portion the 'ergatoplasmic' granules of yolk have previously separated and collected in 'balles vitellines.' This second form of yolk stains distinctly with eosin. The cytoplasmic-like yolk occupies the periphery of the capsule, sending a prominent lamella inward between the embryos—now in process of segmentation—and secondary "parois alvéolaires plus où moins complètes autour des masses éosinophiles." With its 'migratory' nuclei the cytoplasmic yolk stands as a syncytium surrounding embryos and 'eosinophile' yolk. Hallez is of the opinion that these migatory nuclei are effective in changing the nature of the yolk in the 'balles vitellines.' To carry out this function they migrate inward from the periphery.

He finds that the cytoplasm surrounding one of these migratory nuclei makes its way into the substance of each vitelline sphere, eventually drawing with it the nucleus. Later many of these nuclei again emigrate and may then enter into the formation of the ectoderm or of the primary intestine.

Thus it is to be borne in mind that, if Hallez's interpretation be correct, both the entoderm and at least a part of the ectoderm cells in the definitive embryo contain nuclei and cytoplasm which have undergone little change since leaving the vitellaria; in other words, the relationship of these cells to those derived from the egg is apparent only when one recalls that ovary and vitellarium were at their beginning differentiated from the same mass of cells.

Bresslau ('04) traced the history of the yolk cells in Mesostomum ehrenbergi, M. productum, M. lingua, Bothromesostomum personatum and Plagiostomum girardi. The yolk cells of the former behave like those of P. cardii in that they soon separate into two groups. The outer ones become flattened so as to form a shell membrane between the capsular shell and the inner yolk cells. The latter become vacuolated to such a remarkable extent that the nucleus and a small amount of cytoplasm is finally flattened in the form of a thin cresent on one side of the vacuole As the ectoderm formed from the anterior portion of the developing embryo envelops these yolk cells, the nuclei of the latter gradually degenerate, but may still be discerned between the larger vacuoles after the ectoderm has closed over the posterior end of the embryo.

No such shell membrane is formed in the capsule of Bothromesostomum personatum; here the much more rapid degeneration of yolk nuclei is accompanied by the appearance of multitudes of vacuoles of various sizes, all much smaller than those described for M. ehrenbergi. The formation of the ectoderm and enclosure of the yolk globules within the embryo is essentially the same as in the latter species.

The development of M. lingua and M. productum is so similar that Bresslau figures only the former. While a limited number of yolk-cell nuclei come to lie next to the shell the others rapidly disintegrate. No distinct shell membrane is indicated

The newly formed capsules of the Alloiocoele, Plagiostomum girardi, are stated by Bresslau to contain from ten to twelve eggs distributed amongst several hundred yolk cells. The latter then arrange themselves in groups about the several eggs. In the behavior of the yolk-cell contents a notable difference is to be observed in comparing it with the process of yolk transformation in Mesosotomum. The vitelline substance consists of a large number of small refractive spherules which collect at the periphery of the cells. Gradually the cell membranes disintegrate, the process setting in first next to the egg. Since the nuclei, like those in M. ehrenbergi, remain distinct for some time a yolk syncytium results. Vacuolization does not occur until after the embryonic ectoderm has begun to grow out over the yolk. As in three of the four species of Mesostomum a small number of yolk-nuclei become flattened on the surface of the vitelline mass, while the remainder, although less conspicuous than at first, remain visible, scattered through its interior until the ectoderm has enveloped the yolk. Here again no shell membrane is developed form the yolk cells.

For the Dendrocoeles, Planaria torva, P. polychroa and Dendrocoelum lacteum, Mattieson ('04) found that about one hundred and fifty of the yolk cells immediately surrounding the egg become separated from those farther removed and pass over into a syncytium. This change takes place before the enclosed embryo has reached the two-celled stage. Mattieson concluded that, even previously to the formation of the syncytium, an active interchange of material between the egg and yolk cells takes place. The nuclei of the yolk cells which enter this syncytium slowly degenerate, but are still met with at the stage when the pharynx is differentiated.

Two ectoderms are developed in these Dendrocoeles. First a transitory epithelium is formed by blastomeres which migrate through the yolk and arrange themselves in an extremely thin sheet over the surface of the yolk syncytium. Later the definitive ectoderm, arising also from derivatives of the blastomeres, is laid down beneath the first.

To summarize: Bresslau ('04) observed the development of a distinct shell membrane about the yolk mass in the capsule of Mesostomum ehrenbergi. It was derived by a differentiation of the outermost vitellarial cells. In Mesostomum lingua and Plagiostomum girardi he found occasional nuclei lying in small masses of cytoplasm at the periphery of the yolk spheres, while in the case of Bothromesostomum all of the yolk nuclei degenerated; none showed any tendency to form a membrane at the surface of the vitelline mass. On the other hand Mattieson ('04) determined that a 'primary ectoderm' was established about the yolk-syncytim of Planaria torva, P. polychroa and Dendrocoelum lacteum by the union of migatory blastomeres.

According to Hallez's interpretation, certain of the yolk-cell nuclei in Paravortex cardii not only form a peripheral syncytium outside the embryos and the nutritive portion of the yolk, but later enter into the formation of the permanent ectoderm as well as the primary intestine.

The behavior of the yolk cells enclosed in the capsule of P. gemellipara resembles that in the case of Bothromesostomum personatum in that all their membranes immediately disintegrate. The process is so rapid that in two hundred and seventeen worms sectioned only one capsule (fig. 11) was encounted where a considerable part of these cell membranes remained intact. As in Bothromesostomum also, the yolk-cell nuclei at once begin to degenerate. Ordinarily they have disappeared altogether by the time cleavage is well under way, but occasionally degenerate masses of chromatin embedded in small islands of nucleoplasm remain visible until the primary entoderm cells have been differentiated. No membrane or temporary ectoderm is formed by the peripheral yolk cells.

The yolk in perserved material, whether enclosed within a cell membrane or lying free in the capsule, consists of multitudes of small globules and flakes lying in a clear plasma. On the whole, the appearance of the yolk in a newly-formed capsule is very similar to that in the vitellaria. Since in living yolk all particles exist as either spherical, oval or oblong granules (figs. 12, 15) it is probable that the flakes present in preserved material are globules exploded by the action of reagents.

The nuclei of the cells shown in figure 11, whose membranes still remain intact, are already beginning to disintegrate. Succeeding stages of degenerating yolk-cell nuclei appear throughout this section as well as that represented in figure 10.

Occasionally individual yolk cells, perhaps endowed with unusual vitality, retain the membrane until late cleavage in the condition presented when the cell was enclosed in the capsule.

3. Maturation and fertilization

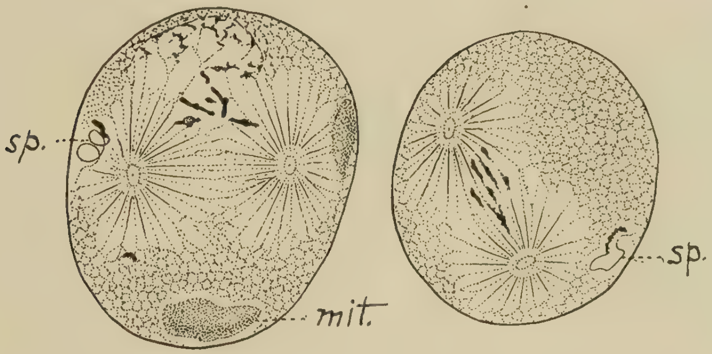
a. Insemination. That the egg in P. gemellipara may be entered by the spermatozoon immediately after leaving the ovary is plainly indicated in figure 9. which shows several spermatozoa between the two eggs and surrounding yolk cells. A careful search through all the sections of these ova yields no evidence that either one has been penetrated.

Each egg of another capsule, illustrated by text figure 4, about which the shell membrane had been formed, contains a spermatozoon. Either insemination has just taken place or the spermatozoon remains quiescent for some time before forming the male pronucleus, for both of these show plainly the long, deeply-stained head and a delicate coiled flagellum.

Without doubt many spermatozoa become enclosed in each capsule, where, as Patterson points out, their identity becomes obscured by the yolk granules. Occasionally a mass of sperm occupies a vacuole in the yolk; such a condition is shown in text figure 5. Presumably the sperm cells continue active in the capsule for some time, so that any egg that is not inseminated at the time it enters the capsule may later encounter the sperm.

Several other eggs have been observed in which the male pronucleus had become lobulated or amoeboid in shape, and had approached, in some a lesser and in others a greater distance toward the female element (text fig. 7).

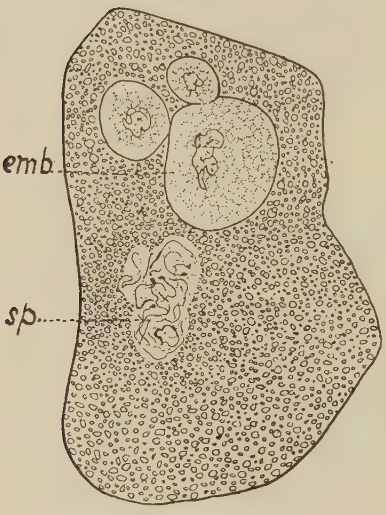
b. Maturation. Patterson described an achromatic figure as appearing in the egg before it leaves the ovary, and remaining until after the formation of the capsule. In his paper ('12) on Graffilla (Paravortex) gemellipara he gave some space to an account of this spindle, noting the remarkable characteristics of its behavior. Although he believed at that time that the spindle later disappeared, he came to the conclusion after a study of the uterine spindle of Planocera that it was not entirely eliminated, but simply contracted to so small a size as to escape easy detection, and was only an early condition of the maturation spindle.



Text fig. 4 Section through two recently fertilized eggs of one capsule. Yolk and capsular membrane not shown. *mit.*, mitochondrial mass; *sp.*, spermatozoon. × 730.

Although occasionally a faint suggestion of a radial arrangement of the granules is perceptible in the yolk-nucleus mentioned above, in all my material no egg shows a true spindle until it has left the ovary and has become surrounded with yolk cells in the distal end of the antrum. It has, of course, for sometime been accessible to spermatozoa. A single egg was observed thus enclosed in loosely massed vitelline cells. It contained a large spindle occupying nearly the entire diameter of the egg. An exact count of the chromosomes in this spindle is impossible on account of poor preservation, but certainly more than the reduced number, four, are present This fact, with the absence of any polar body, stamps it as the first polar spindle. Its appearance thus before the egg has been enclosed in its capsule is believed to be unusual.

The first maturation spindle typically appears after the two ova and their yolk cells have been encapsulated. At the time of its greatest length the spindle occupies nearly the entire polar diameter of the egg (text fig. 8) Each centriole appears as a tiny dot in the center of a large centrosphere, from which radiate the conspicuous astral rays. Owing to the minuteness of the chromosomes, and the extreme difficulty in obtaining all the stages of the process of maturation, it is hard to determine in any given case just what phase they represent. One must depend rather on the presence or absence of polar bodies and



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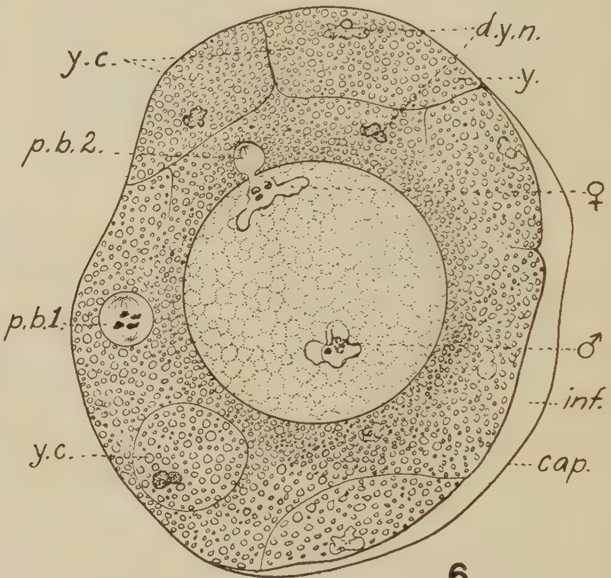

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Text fig. 5 Section of a capsule containing in the yolk a vacuole filled with spermatozoa. *emb.*, embryo; *sp.*, spermatozoa. × 400.

Text fig. 6 Reconstructed section in a plane perpendicular to actual sections through a capsule containing an egg which has given off both polar bodies. *cap.*, capsule; *d.y.n.*, degenerating yolk cell nuclei; *inf.*, infiltration; *p.b.1*, *p.b.2*, first and second polar bodies; *y*, yolk; y.c., yolk cells. × 730.

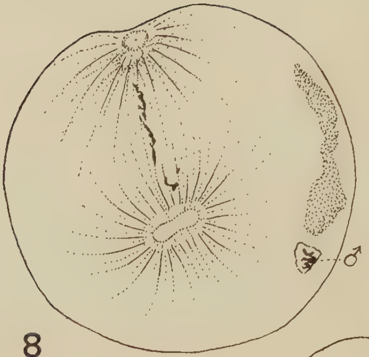
spermatozoon, and upon the number and position of the chromosomes, than upon the shape of the latter.

The egg at the left in the capsule in text figure 4 has extruded no polar bodies, contains a spermatozoon and the chromosomes are just being drawn from the disappearing germinal vesicle. Its mate agrees with it in the first two particulars, but shows an advance in that eight small masses of chromatin, which I conclude to be diads, are present in the spindle; in other words, the cell is in the early metaphase. Text figure 8 pictures a similar condition, except that it is impossible to distinguish all the chromosomes. One centrosphere has already divided, while the other is in the process of division.

Hallez ('09) determined that sixteen 'caryomerites' are present in the first polar spindle of the egg of P. cardi. Eight of these are eliminated in the first polar body. The second maturation spindle contains, as he expresses it, "four chromosomes (= eight caryomerites), half of which enter the second polar body."

My material yielded but a single instance where the first polar body had been given off, and the second maturation spindle was forming. In this egg (fig. 9) the first polar body, not quite detached, has already begun to degenerate, as indicated by the scattered chromatin. The centrosome remaining in the egg has divided to form the second polar spindle whose fibers are drawing the four diads toward the equatorial plate. Another section contains the spermatozoon which has begun to enlarge to form the male pronucleus.

In the preparation which best shows the first and second polar bodies the capsule was sectioned in such a plane that portions of the second polar body, though still connected with the egg by a protoplasmic bridge, lie within two of the serial sections, neither of which passes through the egg itself. A third section, however, contains a thin tangential portion of the egg showing the female pronucleus with small masses of chromatin. Text figure 6 represents a reconstruction of this capsule. The female pronucleus is lying just inside the vitelline membrane in the animal pole of the egg. Lobule formation has again set in.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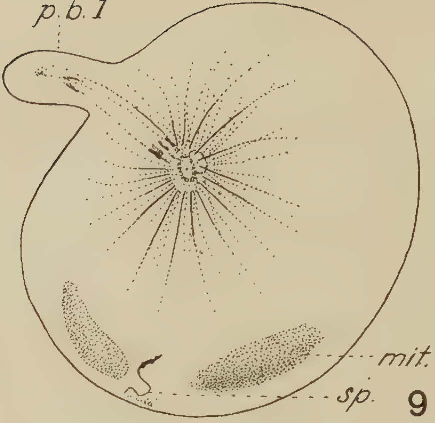
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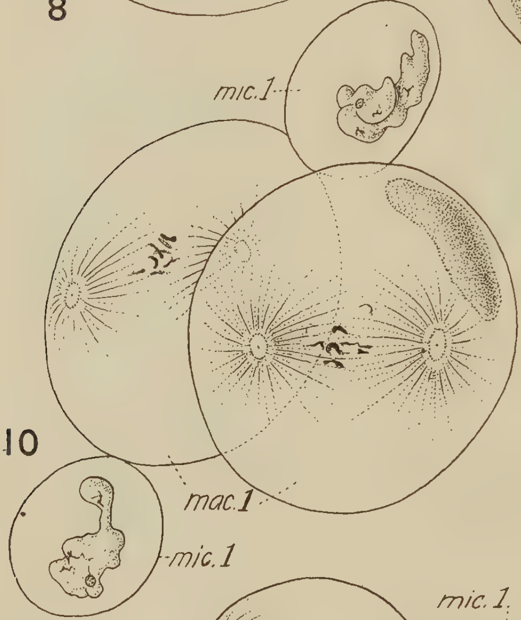
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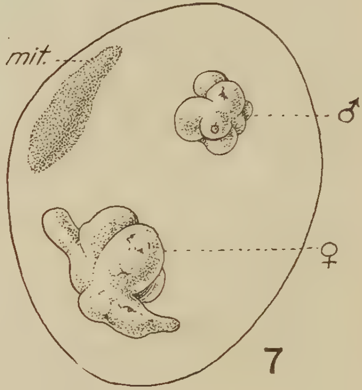
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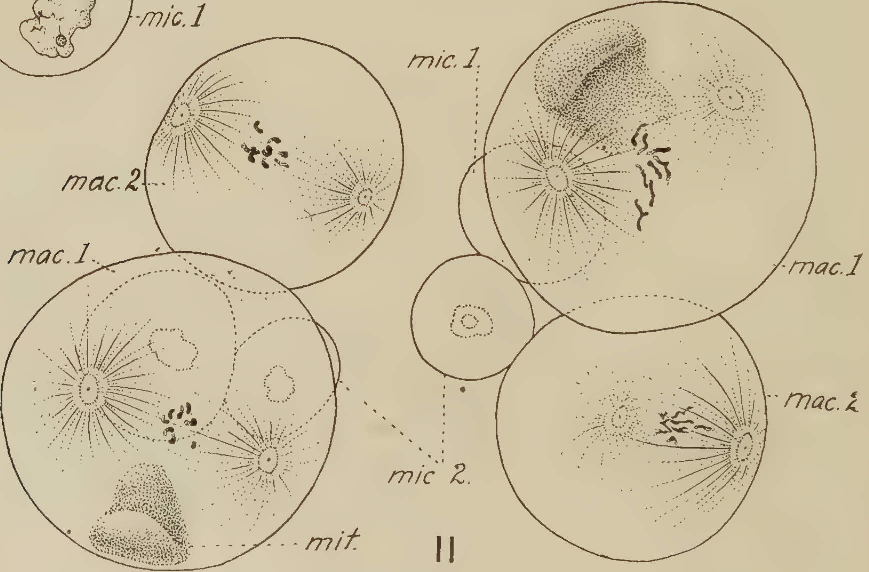
7


mic.1
mac.2
mac.1
mac.1
mac.2
mic 2.
mit.

11

The first polar body at the left of the ovum has approximately twice the diameter of the second. That is it unmistakably smaller than the first two micromeres can be determined by comparing it with the first cleavage figures seen in text figures 10 and 11, while in text figure 12, drawn from life; the polar bodies and first micromere can be directly compared.

c. *Fertilization.* As stated above, insemination occurs before the onset of maturation. The point of entrance, illustrated by Patterson's figures and by text figures 6 and 9 of this paper, is somewhere in the hemisphere opposed to that from which the polar bodies are extruded. During maturation the sperm nucleus, after losing its flagellum, moves toward the center of the egg, at the same time enlarging to form the irregular male pronucleus.

Before their union both pronuclei become curiously amoeboid or lobular in form (text fig. 7). Mattieson ('04) noted this phenomenon in Planaria torva and P. polychroa, and Bresslau ('04) observed it in several Rhabdocoeles. The same was observed by Hallez ('09) in P. cardii.

The male pronucleus in text figure 6, having entered at some point near the vegetal pole, has penetrated the egg for about one quarter of its diameter. A distinct darkly stained mass of chromatin occupies the center of the otherwise chromatinless vesicle. Since, unfortunately, this preparation was rather heavily stained it is impossible to discern with certainty any degenerating remains of the sperm flagellum among the count-

Text fig. 7 Egg showing the lobulated male and female pronuclei. × 730.

Text fig. 8 Thick section of an egg containing the first polar spindle. One centrosphere has divided and the other is in the process of division. ♂ male pronucleus. × 730.

Text fig. 9 Section of an egg from the same capsule as the preceding. The first polar body is just being given off. *sp.*, spermatozoon; *mit.*, mitochondrial mass; *p.b.1.* first polar body. × 730.

Text fig. 10 Reconstruction from sections of a pair of embryos in both of which the first micromere has been given off. *mac.1.* first macromere; *mic.1.* first micromere. × 730.

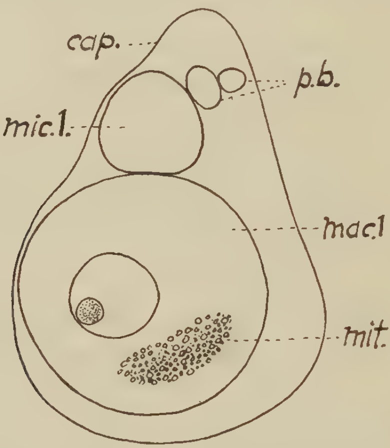
Text fig. 11 A pair of embryos from one capsule, each consisting of two macromeres and two micromeres. *mac.1*, *mac.2*, first and second macromeres; *mic.1*, *mic.2*, first and second micromeres. × 730.

less dark granules in the egg cytoplasm. It is plainly seen, however, in the earlier stages illustrated in text figures 4 and 9.

I can furnish no evidence concerning the union of the pronuclei; the most careful search failed to disclose any trace of centrosome and aster associated with the male pronucleus.

4. Cleavage

In the study of early cleavage in living capsules a serious difficulty has been encountered in the thick bed of yolk which surrounds the embryos. Text figure 12 was drawn from an egg, which, contrary to custom, had entered the antrum, become surrounded by the capsular membrane, and, since no yolk cells were enclosed, was conveniently easy to observe. If an attempt be made to remove the embryos at this stage they fragment. Late blastulae may be thus removed with some success (fig. 15).



Text fig. 12 Outline from life of a young embryo observed in the antrum of the mother. *cap.*, capsular membrane; *mac.1*, first macromere; *mic.1*, first micromere; *mit.*, mitochondrial mass; *p.b.*, polar bodies. × 730.

The only alternative was a trial of various stains. As yet I have not had much success in staining the embryonic cells and at the same time leaving the yolk sufficiently transparent. Neutral red and methyl green were used to stain *in vivo*. Even a dilute solution of the former acts very quickly on the yolk granules, so that no clear idea of early cleavage was gained by its use. Methyl green, a stain for living nuclei, left the yolk too opaque to allow any satisfactory determination of its effect on the embryo beneath. Acetic carmine and methyl blue, besides killing the embryo, again stain the surrounding yolk too strongly.

In later stages of development, after the embryos have migrated to the surface of the yolk or have taken it into their bodies, all the stains mentioned above can be used to advantage.

It is evident then that we must depend upon sectioned material for observations of the progress of cleavage and gastrulation. More or less uncertainty inevitably follows from the various degrees of contraction which the capsules undergo during fixation. (Hallez was partially successful in avoiding contraction by first placing the worms in sea water containing a little chloral-hydrate or cocaine.) The effect of displacement upon the embryonic cells is, of course, greatest in early cleavage. Earlier investigators have noted that particularly in Turbellarian development there is a very weak affinity between the neighboring blastomeres. Bresslau ('04) shows clearly in Plagiostomum girardi and that the blastomeres normally separate for distances often equal to their diameters; this phenomenon is evident as late as the twenty-four celled stage. Hallez's figures show in Paravortex cardii also a loose group of blastomeres. Later the cells draw together to form at first a rather loose, then a compact mass.

So in the case of P. gemellipara, although the blastomeres lie normally in fairly close apposition to one another, their mutual bonds are easily broken by outside pressure. As a result, sections often reveal isolated blastomeres crowded for some distance into the surrounding yolk.

For the reasons above noted it seems inadvisable to attempt any detailed investigation of cell lineage in this organism. Certain major characters, however, can be consistently observed.

a. Micromeres and macromeres. The first cleavage division of the egg of Paravortex gemellipara, as of P. cardii, gives rise to a pair of cells between which there is a marked disparity in size. Text figure 10, a reconstruction of the two embryos of one capsule, shows very well the relative proportion in size of the first micromere to the macromere. It also indicates that gravity may not be sufficient to determine from which pole of the egg the micromere shall arise, for in this capsule the polarity of one egg is exactly opposed to that of the other. Not too much reliance, however, is to be placed upon the polarity as indicated in sectioned worms. When the animals are dropped into the killing fluid the tissues are as likely as not to become fixed while the specimens are inverted. Hence if the embryos rotate with respect to gravity, their positions with respect to the mother might easily be changed while the fixing fluid was penetrating her body-wall and parenchyma. But since both embryos are equally free to rotate, the evidence afforded by text figure 10 suggests that gravity does not wholly determine the embryo's polarity. Indeed, this figure, taken in conjunction with figures 14 and 18, indicates that the opposed polarity invariably exhibited by the two embryos of a capsule in later stages is already determined at the time of the first cleavage of the egg.

According to Patterson's account the first cleavage of the fertilized egg of P. gemellipara results in the splitting off of a micromere of such a size that it is necessary to count the chromosomes in order to distinguish it from the first polar body. We find, however, (text fig. 12), that this micromere is at least twice as large as the polar body, and furthermore (text fig. 10) that its nucleus immediately enters a resting stage during which it may be more or less amoeboid. No such nucleus has been found in the polar bodies. Owing to the rapidity with which the latter disintegrate, it is often impossible to find both the first polar body and micromere present together so as to compare them directly.

It is characteristic of the blastomeres of Paravortex gemellipara, as of P. cardii and other Turbellaria, that the nuclei are strikingly vesiculated.

Hallez asserts that in P. cardii the first micromere divides before the first cleavage of the macromere. Text figure 10 of this paper would indicate, on the other hand, that, since in both embryos the micromere contains an amoeboid nucleus while the macromere shows a spindle, the latter is about to divide before the first undergoes division. The second cleavage of the large macromere, judging from the conditions represented in text figure 11, results in the formation of two smaller macromeres somewhat unequal in size. The positions of the spindles which are inaugurating a further division of the two macromeres of both embryos indicate that the cleavage is to be of the spiral type described by Bresslau for Mesosotomum ehrenbergi. He found, however, that there were three micromeres present before the second macromere was cut off. Three of the macromere spindles in this pair of embryos are in the prophase, and clearly show eight chromosomes being drawn into the equatorial plate. On account of an artifact in one section of the fourth macromere the arrangement of the chromosomes could not be determined.

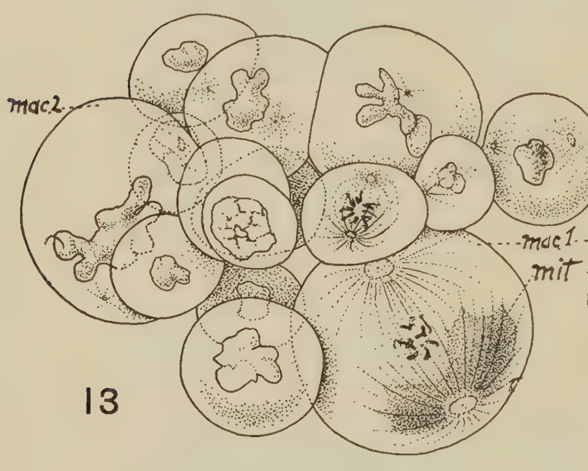
It is important to notice that during early cleavage the mitochondrial mass lies apparently inert at one side of the macromere and entirely outside the spindle. Not until later does the mass itself become divided.

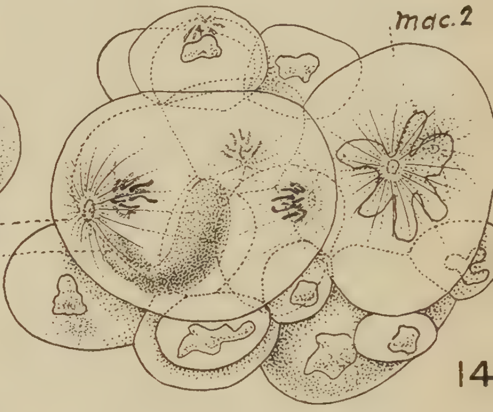
Hallez determined the number of chromosomes present in the equatorial plate of the cleavage spindle of P. cardii to be "four (= 4 groups binaires)." In P. gemellipara, however, the equatorial plate always contains eight U-shaped chromosomes; in the telophase each pole of the spindle likewise holds eight (text figs. 13, 14).

Beyond the four-celled stage cleavage proceeds irregularly as observed by Bresslau, Hallez and others. One can determine, however, that at one pole of the embryo the micromeres become more and more numerous; two macromeres occupy the other pole.

While the macromeres give rise by division to some of the larger micromeres, the latter redivide again and again.

The embryos represented in text figures 13 and 14 contained respectively fifteen and seventeen blastomeres. Text figure 14 is the more normal in that the group of cells is compact without intercellular spaces. The embryo illustrated by text figure 13 had probably been subjected to a considerable degree of contortion. It is noticeable that the blastomeres in well preserved material are closely in contact with yolk, while in shrunken preparations wide spaces intervene; consequently the blastomeres are apt to become displaced. In both figures the pair of larger cells is prominent. The mitochondrial mass still remains in one of the macromeres. Text figure 13 shows it in the form of a hemisphere held at one pole of the spindle, while in text figure 14 it lies, cresent-shaped, outside the astral fibers.





Text fig. 13 Reconstruction from sections of an embryo of fifteen blastomeres. *mac.1*, *mac.2*, first and second macromeres; *mit.*, mitochondrial mass. × 730.

Text fig. 14 Reconstruction from sections of an embryo of seventeen blastomeres. × 730.

b. Behavior of the mitochondrial mass. Although a careful search was undertaken, no cleavage figure was found in which the mitochondrial mass was first being divided. Between the stages shown in text figure 13 and in figure 20 no intervening step was discovered. However, the condition at a later stage, figure 19, suggests that, as the membrane is drawn inward during cleavage of the macromere, the mass is too large to remain wholly in one cell and is so constricted that part is handed on to each of the daughter cells. It is possible, on the other hand, that the division is effected directly by the traction of the spindle fibers upon the mass. Very likely both factors act simultaneously.

Certain it is that beyond the stage where the mitochondrial-mass appears in two or more blastomeres the centrosome lies in its center. Previously the centrosome was difficult to detect among the numerous granules in the cytoplasm, but now that it has come to lie in a more finely homogeneous substance it is conspicuous. Figures 14 and 16 plainly show it in a clear region at the center of the mitochondrial mass. Had its history not been carefully traced the picture here presented would suggest that the whole structure answered to the centrosphere containing a centriole. That the black dot represents more than a mere centriole is indicated by its further behavior. Figure 19 shows that the solid dot becomes enlarged as the new spindle forms, and contains a light center. Ultimately the body assumes the form of a sphere whose shell consists of a multitude of tiny granules, and at whose center lies the true centriole. Furthermore, it lies outside the mitochondrial mass in one cell of figure 20.

c. *Blastula.* It has been noted that no distinct segmentation cavity is formed in the blastula. The conditions at this stage are well shown in figures 14 and 16 which represent sections of different embryos, and in figure 15, a drawing of a living embryo which, with a small mass of yolk granules, was freed from its capsule. The blastomeres lie in rather close contact with each other, with the exception that a cap of cells which have received the portions of the original yolk-nucleus tends to become separated from the mass of embryonic cells which is to give rise to the living tissues of the embryo. A solid morula is, then, typical of P. gemellipara as of Turbellaria in general and many other lower invertebrates. But for the cells containing the mitochondrial mass this morula closely resembles that of Hydra and other Coelenterates. Bresslau figures for Mesosotomum ehrenbergi a morula somewhat less compact and more irregular in outline.

His figures of the morulae of Bothromesosotomum personatum and Mesostomum lingua are similar, with slight characteristic variations. Although the blastomeres in the segmentation of the egg of Plagiostomum girardi separate remarkably from one another, Bresslau finds them later drawing together to form a compact mass.

The morula of P. gemellipara lacks the marked bilateral symmetry due to a sagittal cleft which, according to Bresslau, characterizes a corresponding stage in the development of Mesostomum ehrenbergi. In P. gemellipara it has been found impossible to distinguish the dorsal from the ventral surface of the morula. The point of view in figure 15 is from almost directly anterior; any attempt to distinguish here between dorsal and ventral is clearly of no avail; the cells are all alike. On the lower left, however, the posterior primary entoderm cells are differentiated by their larger size.

d. Homologues of the germ layers. While it is true that, as Bresslau says of Mesostomum and other Turbellaria, no definite germ layers are formed in these worms, at least rather distinct germ regions are discernible. As the development of the morula progresses there are produced as derivatives of the one macromere about sixteen blastomeres which receive a portion of the mitochondrial mass. They form a cup-like layer, one cell thick, over the surface of the morula which lies toward the center of the yolk-mass, and serve, as will soon become clear, to indicate the polarity of the embryo; this cap of cells is posterior to the rest of the morula, and is to be looked upon as constituting the primary entoderm. Two other regions can be distinguished in the morula at this stage, (fig. 16), first, the mes-ectoderm consisting of smaller cells which are conceived to be direct descendants of the micromeres, and second, a layer of larger cells with less dense cytoplasm lying between the primary entoderm and the mes-ectoderm. These are derivatives of the second macromere, seen in text figures 11 and 13, which lacked the mitochondrial mass. Since they ultimately give rise to the intestine it is appropriate to call this layer of cells the secondary entoderm.

In the slightly older embryos represented in figures 17 and 18 the secondary entoderm cells are still actively dividing, but most of them are larger than the anterior mes-ectoderm cells.

5. Absorption of yolk by the primary entoderm

Toward the end of the morula stage a remarkable process of nutrition is inaugurated. As far as has been determined from the literature no closely similar process has been described as occurring in the nourishing of any embryo. The primary entoderm cells, which were seen in figures 14 and 16 already to have but a loose connection with the main embryonic mass, now apply themselves like amoebae to the surface of the yolk which surrounds the posterior half of the embryo. The firmness of their attachment is evident in figure 17; while the embryos have shrunken away, most of the yolk entoderm cells remain closely in contact with the yolk.

It is the function of these cells to absorb a large part of the yolk mass brought into the capsule inside the vitelline cells. The membranes of the latter have all disintegrated, leaving the yolk flakes and granules free in the capsule. Inspection of the living capsule shows the particles in vigorous Brownian movement. There is, therefore, no obstacle to the action of the entoderm cells. They are shown in figure 17 plainly acquiring the yolk particles. Their nuclei are large, distinct, and according to the rule in active cells throughout this animal, markedly lobulated.

The nature of the yolk granules suffers little change during the process, for those inside the entoderm cells have the same form and color as those lying just outside the cell membrane. It is to be noted that all exist as spherules; no flakes such as constitute a considerable portion of the free yolk are at first seen in the entoderm.

The gradual increase in size of the entoderm cells, while ingesting the yolk on the median side of the embryo, at the same time forces the latter outward until that portion of it which from now on is to be recognized as the anterior end comes to lie in contact with the wall of the capsule (fig. 17). This migration of the embryo from an interior to a peripheral position is a step characteristic of all the species considered in this paper. According to Bresslau the embryonic cells migrate to the ventral surface of the yolk in the species of Mesostomum and Plagiostomum which he studied. Hallez ('09) finds it assuming an antero-ventral position in Paravortex cardii. The same migration was observed in the Dendrocoeles by Mattieson ('03) and in the Triclads by Jijima ('84). The embryonic mass of Paravortex gemellipara, like that of P. cardii, is forced to the anterior side of the yolk.

There is considerable variation in the amount of yolk absorbed by the primary entoderm (compare figs. 18 and 20). Hence there remains a larger or smaller quantity disposed, as seen in a median section (fig. 17), in the form of a bi-concave lens between the two embryos. The fate of this mass will be discussed in the following section.

Some of the entoderm cells seen in figure 21 have already reached the limit of their absorptive capacity, while others are still comparatively small. It should be pointed out, however, that there is a great variation in size at the time they are taken into the embryo. That the quantity of yolk absorbed depends rather upon the activity of the cell than upon the amount of yolk in its neighborhood is suggested by the fact that small primary entoderm cells are enfolded by the gastrulating embryo even when there was plenty of material present, as indicated by the quantity later found in the ectoderm.

At the completion of the yolk absorption, and before gastrulation, degeneration of the nuclei of the primary entoderm cells sets in. As represented in figure 21, their chromatin becomes less distinct, while the nucleoli disappear. Finally, with the dissolution of the nuclear membranes, there remain for a time only indistinct vestiges of nucleoplasm with a·little chromatin. Rarely does any trace of the nucleus appear in the cell after it has been taken into the body of the embryo. The activity of the cell having ceased, the presence of the nucleus is no longer necessary.

6. Ingestion of the yolk-gorged primary entoderm cells by those of the secondary entoderm

A remarkable event now happens. Some of the derivatives of the secondary entoderm cells pointed out in figures 14 and 16 become amoeboid and make their way around and between those of the primary entoderm until they come to lie, in some cases, next to the free yolk. Three of these are visible in each embryo of figure 18. Though the nuclei are always prominent, it is only in well-preserved and favorably stained material that the cytoplasm can easily be distinguished. In such cases there can be no question of a syncytium here. Each cell is a unit.

Another amoeba-like character is now manifested by these cells; each primary entoderm cell is ingested by a secondary entoderm cell. Referring again to figure 21 we find that the section passed through the nuclei of several secondary entoderm cells, while the cytoplasm of each stretches in a thin sheet over the surface of the primary entoderm cell which is being engulfed. There is in this embryo no certainty that the cytoplasm has yet completely surrounded the yolk-filled cells. That this occurs later will become clear. It is evident that the nuclei of the primary entoderm cells do not entirely disappear until after ingestion.

7. Gastrulation and absorption of yolk by the ectoderm cells

If by the process of gastrulation is meant the infolding or overgrowth of the entoderm by the ectoderm, then a true gastrulation occurs in Paravortex gemellipara. Bresslau ('04) in describing the development of Mesostomum thought the term inapplicable. It is used in this paper as expressing the process at least homologous with gastrulation.

The first step consists in the differentiation of the ectoderm. The outermost cells of the mes-ectodermic mass at two points (or perhaps in a ring) on the anterior surface of the embryo now become flattened. Between these two points lies a pair of cells which are to give rise to the lining epithelium of the pharynx (figs. 22 and 23). Just as Bresslau finds in Mesostomum so in P. gemellipara the ectoderm cells stretch backward in a sheet over the underlying mass (fig. 21). Figure 22, drawn from a section so thick that only the upper third of the embryo was removed, represents the condition just before the close of gastrulation. The five yolk cells, three of which show the large nuclei and thin cytoplasmic sheets of the secondary entoderm cells whose food they have just become, are being folded into the embryo by the overgrowth of the ectoderm cells. At the right three of the latter are of such a character that they resemble the yolk cells just described.

It is apparent from the above account that gastrulation in P. gemellipara is brought about by a process of epiboly or overgrowth of the entoderm by the ectoderm cells. The latter are formed by differentiation of the external portion of the mesectodermic mass, whereby the cells, first at the anterior end, then gradually those lying more and more laterally, become flattened and push backward over the entoderm.

At this point another phase in the nourishing of the embryo is observed. It was noted above that, after the gorging of the primary entoderm cells, more or less yolk remained in the capsule (figs. 18 and 21). The ectoderm cells, now undertaking the role of nutrition, absorb this free yolk. In figure 22 the cells on the right still show the yolk granules distinctly, indicating that the process here is similar to that carried on by the entoderm. Very soon, however, the yolk undergoes a change. The flakes and granules in each cell flow together into a single large globule. It is evident from this figure that the posterior cells take in most of the yolk, those at the anterior end coming in contact with only a very little. The nuclei of the ectoderm are characterised by the presence of large and distinct nucleoli.

It is strikingly evident that in embryos of corresponding stages of development, particularly that shown in figure 24, the ectoderm yolk-content of some is decidedly less than of others. The conclusion is that the primary entoderm absorbed the greater part. According to this hypothesis but little yolk would have entered the ectoderm cells of the embryo pictured in figure 18. Such individuals as those in figures 22 and 23 must previously have had left over after the absorptive action of the entoderm an amount of free yolk comparable with that shown between the embryos of figure 21.

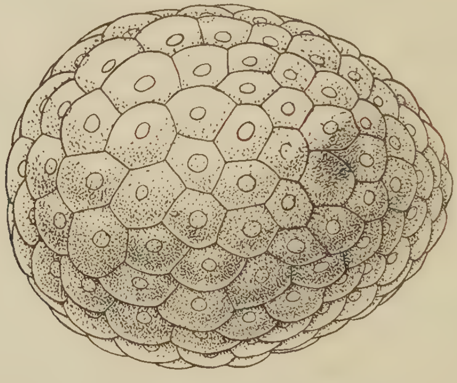
a. The embryo at the close of gastrulation. The picture presented by the embryo at the close of gastrulation is shown in median frontal section by figure 23 which was drawn from an Ehrlich's haematoxylin-eosin preparation. Sections of seven primary entoderm cells occupy the greater part of the young worm, each lying inside an amoeboid secondary entoderm cell. No clear regions have yet appeared in the interior of the former; the yolk globules are evenly distributed.

A similar condition exists in the ectoderm, although a slight advance is evidenced by the tendency of the small globules to fuse into larger masses. The posterior cells are so engorged that their membranes are stretched almost to the point of rupture. On the other hand the anterior ectoderm cells are flattened and contain only a few yolk granules. It is probable that certain large nuclei just beneath the ectoderm in this region are soon to be added to it as parts of new cells.

The formative mass from which are to be derived all the definitive organs lies toward the anterior end of the embryo. Two lines of cells, however, are pushing posteriorly beneath the ectoderm and the primary entoderm. Certain of these are undergoing mitosis, while a few differ from the majority in possessing lighter cytoplasm and definite boundaries. These are regarded as secondary entoderm cells which did not migrate outward to ingest the yolk-laden cells, but were reserved to take a part in the formation of the intestine.

It is impossible in the section from which figure 23 was drawn to detect cell membranes between the smaller nuclei which characterize the greater portion of the undifferentiated mass. One might well regard it as a syncytium were it not that the membranes are discernible in figures 18 and 21 which represent slightly younger embryos, and in figure 24 of one somewhat farther advanced. These three figures are from preparations stained with iron-haematoxylin and eosin after fixation in Flemming's solution.

To conclude the description of the stage seen in figure 23, the pair of cells mentioned above (fig. 22) as being reserved during the early period of ectoderm differentiation for the development of the internal pharyngeal epithelium, are conspicuous at the anterior end of the embryo. Their cytoplasm remains consistently lighter and clearer than that of the adjacent interior cells. Their resemblance is rather on the side of the ectoderm.



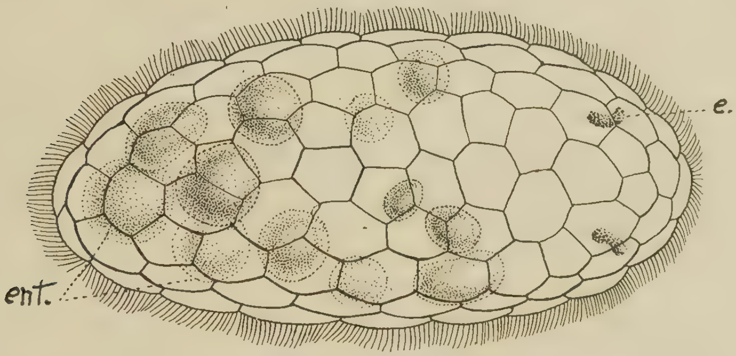
Text fig. 15 Surface view of an embryo forcibly freed from its capsule shortly after gastrulation. Anterior end at the right. × 530.

Figure 25, drawn from a fresh capsule, shows the appearance of a living embryo at a stage slightly later than that in section (fig. 23). Through the transparent ectoderm can be seen the prominent yolk-filled cells of the primary entoderm. The highly refractive bodies so conspicuous in the ectoderm cells are regarded as globules of the same nature as those constituting the free yolk still remaining in the capsule. The larger opaque masses in the posterior ectoderm cells are the homologues of those noted above in figure 22, i.e., masses of yolk undergoing digestion.

Between the capsular shell and the enclosed embryos and yolk granules is a clear zone filled with a colorless fluid. Probably a large part of this clear region is occupied by a fluid of a watery nature which has been absorbed from the parenchyma. It was pointed out above that capsules which are freed in sea water immediately swell on account of pressure due to water absorption.

8. *Organogenesis to the time of birth*

a. Origin and development of the eyes. The first organs to be differentiated from the mes-ectodermic mass in Paravortex gemellipara are the eyes. Although at first this seems rather remarkable, still it is reasonable to expect that, since the major portion of each definitive eye consists of a single cell, this cell must at an early stage take on a different appearance from those about it. Already in figure 24 the eye cell shown has become distinct by its larger size and clearer cytoplasm.



Text fig. 16 Surface view of an embryo which of its own account had made its way into the mother's parenchyma. *ent.1.*, digested remains of yolk in primary entoderm cells; *e*, eye. × 400.

These eye cells appear, one on either side toward the dorsal side of the undifferentiated mass. That seen in figure 24, an oblique frontal section, is an individual cell with a distinct membrane (Hallez was unable to distinguish the membrane in P. cardii) and nucleus. The latter at first lies in the center of the cell, but soon assumes an eccentric position toward the posterior median side. The presence of only a single melanin granule is certain on account of the fact that the whole cell is included in this section. Moreover, the second eye of this embryo was in the same stage.

The fate of these undifferentiated cells will be treated later. The eyes are at first separated from the ectoderm by three or four rows of cells. Partly as a result of increase in size which the eyes undergo, and partly because the cells among which they lie are gradually withdrawn to form other tissues, the eye cells shortly assume a position so close to the ectoderm that only one layer of nuclei intervenes. The left eye in figure 28 (from a frontal section through the eyes and brain) has become finally located. Apparently at this stage the optic cells are entirely independent of the brain. Unusual turgidity characterises the embryonic eye; it retains a spherical form in spite of the pressure which must be exerted upon it by the surrounding cells.

A series of stages in the development of the eye is illustrated by figures 30 and 31. All were drawn with the aid of a camera lucida in order to bring out the gradual increase in the size.

During the period of greatest activity of the eye cell its nucleus is prominent, with a fine chromatin reticulum; a nucleolus has not been detected. It has been mentioned in the section dealing with the cleavage of the egg that the active blastomere nuclei tend to assume an amoeboid form. So in the eye cell the nucleus becomes to a lesser extent irregular during the deposition of melanin granules.

Two types of melanin spherule deposition have been observed. The more usual is that wherein the granules arise one at a time. While those first formed are increasing in size new ones appear in the cytoplasm (fig. 30). The earlier melanin bodies are formed close to the lateral surface of the nucleus. Hence it might be inferred that, being laid down under the influence of the latter, these would soon be pushed outward by new ones to be formed in the same proximity to the nucleus. Such a sequence, however, has not been observed; the new spherules arise as often on the outside of the group as on the inner nuclear border. Indeed, after the process has gone on for some time practically all the smaller granules are seen to lie on the side opposite the nucleus.

As to the general arrangement exhibited by this group of melanin spherules, the early irregularity soon gives way to that of a cup whose concavity faces outward (fig. 30 *d*, *e*). Consequently a meridianal section is crescentic. The floor of the cup, lying against the nucleus, consists of from two to four rows of larger granules, while as the rim is approached the thickness is gradually reduced to one small sphere.

According to the second type of melanin deposition many minute particles arise simultaneously in the cytoplasm between the eccentrically placed nucleus and the outer membrane of the eye cell. Figure 31 represents the appearance and history of these granules. Since the final arrangement is the same as in the eye at the end of the series described above, it appears either that the spherules nearest the nucleus increase more rapidly in size while those at the margin of the cup remain consistently smaller, or that new granules have subsequently been added to this rim. The latter hypothesis is supported by the fact that a larger number of spheres is present in the definitive eye than was first seen to arise.

It seems clear that the pigment granules arise in the cytoplasm, probably under the influence of nuclear energy. Their arrangement and appearance are not visibly affected by the method of preparation; hence they can be favorably studied in preserved material. When first detected each is a tiny homogeneous refractive body whose form is that usually assumed by a drop of fluid whose surface tension properties are different from those of the fluid in which it is suspended—in this case cytoplasm. There is no evidence that these spherules enlarge by the accretion of successive layers of a more solid material. The conditions rather favor the idea that they grow in the manner observed in the case of fat drops, i.e., by an accumulation of more and more fluid.

With the increase in size the yellowish color deepens to a reddish brown, and a darker central dot appears in each granule (fig. 30 *c*). From this point on there is little to be noted beyond the increasing opacity due to the greater amount of material. Finally, when the pigment spheres have reached their full size their color is an intensely dark brown (fig. 30 *e*). At low magnifications the pigment cup is to all appearance black.

The nucleus of the eye pigment cell is gradually forced by the melanin granules against the membrane at the postero-median side. Figure 32 shows it thus flattened. In proportion to the size of the cell the nucleus is now smaller than during the period of its greatest activity.

b. Development of the brain and larger nerve trunks. Bresslau ('04) determined that the formative mass in the embryo of Mesostomum ehrenbergi soon separated into three anlagen, a large antero-ventral mass, consisting at first of two ganglia which later formed the brain; a spherical portion just posteriorly from which the pharynx musculature arose; and third a smaller cresentic gonad anlage.

Hallez ('09) noted the similarity exhibited to this stage of Mesostomum by the corresponding stage of Paravortex cardii. In the development of P. gemellipara the differentiation of the brain, pharynx musculature, and gonads is essentially the same as in the species studied by Hallez. In two laterally symmetrical regions toward the dorsal side of the anterior embryonic mass a proliferation and grouping of nuclei occurs soon after gastrulation. Thus the two ganglia of the brain arise. While the nuclei become more and more numerous, it has been impossible to discover any mitoses in this region, a fact which led Hallez to suggest that division here is amitotic.

The second feature noted in the development of the brain is the appearance simultaneously of two clearer protoplasmic areas in the center of each lateral ganglion. The substance occupying these two regions has been called 'Punktsubstance' by Leydig. As pointed out by Bresslau and Hallez, these two centers soon fuse to form a transverse dumb-bell shaped core on the outside of which the ganglion cell nuclei are arranged. The latter are at first indistinguishable from those of the surrounding portions of the undifferentiated cell mass, but in slightly older stages are visibly smaller (figs. 29 and 53).

Concerning the 'Punktsubstance' mentioned above one characteristic is to be noted that neither Bresslau or Hallez described in either text or figures, namely, the striated or fibrillar structure of the lighter brain core when viewed in sagittal sections. That these striations are to be regarded as the fibers or prolongations of the ganglion cells whose nuclei are distributed over the surface of the brain, is clear from the works of many investigators. These structures are well represented in Bronn's Klassen und Ordnungen des Thier-Reichs. Obviously it is these fibers which, in a cross-section of the brain, i.e., a sagittal section of the worm, are responsible for the dotted appearance. Figures 29 and 37 are of sections so cut that some nerve fibers are seen in cross and others in longitudinal section.

Little remains to be added in regard to the nervous system. Since the material was not treated with differential nerve stains, it is extremely difficult to follow the nerve trunks through the mesenchyme, even in older specimens.

Concerning the origin of the two posterior nerves of Paravortex cardii Hallez says:

"Quant aux deux troncs nerveux postérieurs, tout ce que j'ai pu voir, c'est qu'ils se différencient *in loco*. . . ."

It is believed that in P. gemellipara these posterior nerves can hardly be said to arise '*in loco*.' They evidently push backward through the mesenchyme from the posterior angles of the brain. In figure 28 the nerve fibers can be traced thus for a very short distance, but in a later stage (fig. 29) the ends have reached farther toward the posterior end.

It is important to notice that many ganglion cell nuclei are carried backward along the nerve trunks. Consequently the cephalic mass suffers a reduction. From the conditions observed it is concluded that in the Turbellaria, as in the vertebrates, the nerve fibers, with groups of ganglion cells, originate in the region of the brain and migrate toward the periphery.

That the optic nerve trunks have a similar origin may be argued from the appearance presented by figures 28 and 37. However, these nerves may have another origin. The fibrous brain core in figure 28 has begun to stretch forward at two antero-lateral points; a decided advance has taken place at the stage pictured in figure 32. Here the left lobe of the brain and the left eye are viewed from the dorsal surface. The nerve fibers show very plainly passing from the 'Punktsubstance' region of the brain outward and between the ganglion cells. The course of the nerve is then directed laterally around the posterior side of the pigment cell to its external surface.

At this point the eye cell, whose origin and growth have already been described, undergoes an invagination. This is apparently effected by the pressure of a nerve cell which is seen lying in the concavity. This undoubtedly will form the 'sight-cell' or end organ which is stimulated by light rays. A second nerve cell nucleus is observed just posteriorly.

It can now be suggested that the two cells lying close outside the pigment cell in figure 32 arose in this position and have sent fibers backward to meet the anterior extension of the brain. Two facts support this view. First, undifferentiated cells during development lie in this region between the ectoderm and the pigment cell. Secondly, it is well known that in higher forms the cell body of the receptor is located in the retina, while the axone extends into the brain. It is obvious, however, that one must not attempt to homologise too closely the course of development of a turbellarian with that of a vertebrate. In the latter the retinal cells are first carried out by the optic cup from the central nervous system, while in the Turbellaria the boundaries of cell groups in the cephalic region are notably indistinct.

It has proved impossible to discern in my material the structure of the cell or cells which occupy the external invagination of the pigment cell in the adult worm. Since the histology of the eye in related Turbellaria has been determined, it is to be expected that the receptor of P. gemellipara is similar to that observed, for instance, in Phaenocora and Mesostomum as shown in Bronn's Klassen und Ordnungen des Thier-Reichs.

Figure 33 of this paper was drawn from an adult eye of P. gemellipara, and shows that the optic nerve, as in the embryo and in the species to which we have just referred, passes to the lateral invaginated surface of the pigment eye-cell. A nucleus rests close outside, apparently in the nerve.

c. Pharynx. Simultaneously with the differentiation of the brain the anlage of the pharynx musculature becomes marked off from the former and from the remaining tissue by a closer grouping of larger nuclei. This mass lies ventral and very slightly posterior to the brain, but its boundaries are at first so indefinite that, were it not for the simultaneous ingrowth of the cells which form the internal pharyngeal epithelium, the anlage would with difficulty be distinguished.

Considerable attention has been paid by European observers to the development of the pharynx of the Turbellaria. Mattieson in 1904 described the process in the Dendrocoele, Planaria torva, in which the pharynx arises as a spherical mass of cells on the ventral surface just beneath the primary ectoderm. While the outer cells form the musculature of the organ, the internal epithelium arises from a few large cells situated on the interior. Against the inner end of the pharyngeal mass lie four or five cells which in Mattieson's opinion are entodermic in nature. No vestibule intervenes between the pharynx and mouth.

Bresslau ('04) found that in the Rhabdocoele Bothromesostomum personatum the internal epithelium likewise arises *in loco.* In Mesostomum ehrenbergi, a different condition exists; the lining epithelium is derived from a rod of cells which grows inward from the ectoderm and pierces longitudinally the mass which eventually becomes the pharyngeal musculature with its glands. After traversing the pharynx the ectodermic rod acquires a lumen and gives rise at the inner end to what Bresslau terms the inner pharngeal pouch lying between pharynx and yolk, while between the pharynx and mouth an outer pharyngeal pouch has a similar origin.

The main features in the development of the pharynx in Paravortex gemellipara agree with those described by Bresslau for Mesostomum ehrenbergi. Until after the ingrowth of the epithelial rod no definite grouping of nuclei occurs to mark the origin of the pharyngeal bulb. The cells which are to take a part in its formation lie loosely arranged in the mid-ventral portion of the undifferentiated cell mass just beneath and posterior to the brain.

In the description of the ectoderm formation there was pointed out a pair of cells which, although at that time lying at the anterior surface of the embryonic mass, did not enter into the development of the body covering. These two cells, well shown in figure 23, together with two or three others not seen in this section proliferate the rod of cells which, as in Mesostomum, grows inward to penetrate the mass which lies beneath the brain.

An intimate connection between these cells and those of the ectoderm is evident in figures 34 and 26. The former represents a section which is obliquely sagittal. It is evident that before the cells of the pharyngeal bulb have become closely grouped, the posterior cells of the epithelial rod have penetrated the mass of dark nuclei and lie against the entoderm. A distinctly clearer cytoplasm and paler nuclei serve to distinguish them from the neighboring cells. Each of their nuclei, like those of the ectoderm, with whose early history these cells are closely associated, contains a nucleolus. Externally a slight invagination of the ectoderm indicates the future position of the mouth.

A slightly later stage is shown in figure 27, a transverse section through the posterior lobes of the brain and the center of the pharynx. Portions of seven clear cells belonging to the epithelial rod are conspicuous in the center of the mass of nuclei which have now become closely grouped with their cytoplasm in what appears to be a syncytium. For a longitudinal view of the organ at this stage the obliquely frontal section in figure 26 is of interest. The nuclei of the bulb are disposed roughly in two rows, somewhat toward the periphery of the mass. As yet no cell membranes appear between them. The epithelial cells are constricted within the bulb so that they become elongated, parallel with the longitudinal axis of the organ; one cell appears to traverse its entire length. Those between the pharynx and epidermis are irregularly arranged, while at the inner end of the pharynx is a group of five similar cells. Three of these appear to be intimately connected with the epithelial rod; the posterior pair, however, are not thus closely associated, but have, it is believed, an entodermic rather than an ectodermic origin. Further notice of these cells will be taken when the development of the intestine is considered.

In an older embryo (fig. 37) the muscular portion of the pharynx has become sharply defined, although no external epithelium has yet developed. The nuclei have not migrated farther toward the periphery than the point at which they were observed in the preceding stage. A considerable enlargement of the anterior and posterior cells of the epithelial rod has occured. No blind sac, however, is formed, as in Mesostomum, at either end of the pharynx. Indeed, no lumen appears in the pharyngeal apparatus until shortly before the embryo leaves the mother's body.

If at this stage the pharynx and associated tissues of Paravortex gemellipara be compared with the corresponding stage in Mesostomum ehrenbergi it is evident that the development of the pharynx in the former lags behind that in the latter. Mesostomum shows a well defined epithelium bounding a large cavity, the latter consisting of an internal and external pocket communicating by a tube through the pharynx. Bresslau considered the interior pocket as the esophagus, whose cells differed in point of origin from those later forming the intestine. The homologue of this pocket in P. gemellipara is the group of large cells just posterior to the pharynx.

In the cross section of this organ represented in figure 38 an advance is shown in the presence of an external epithelium. A thin layer of plasma containing a few small flattened nuclei covers the pharyngeal bulb. Bresslau derived this tissue from the outermost portion of the pharyngeal musculature anlage in Mesostomum; but in Paravortex the indications point to a mesenchymatous origin, such as von Graff ascribed to it in other Turbellaria. When one remembers that in either case the cells constituting this membrane arose in the undifferentiated cell mass the point resolves itself into one of time.

Concerning the development of the radial, circular and longitudinal fibers which arise in the pharyngeal musculature nothing has been observed in the young of Paravortex gemellipara while still in the mother's body.

d. Musculature. Like the nervous system and the pharynx the mesenchyme and dorsoventral muscle cells arise from the undifferentiated mass which occupies the anterior portion of the embryo at the close of gastrulation. If one compares figure 23 and figure 24 it is noticed that in the latter indifferent cells have migrated posteriorly so that a continuous sheet of them lies between the ectoderm and entoderm. Their amoeboid nature is further evidenced by the extreme delicacy of their membranes. Indeed, it is impossible to distinguish a boundary where two cells come in contact with each other. The tissue at this stage may be called a syncytium. Occasional mitoses are observable in this region. The plane of the section of the embryo at the right in figure 24 is sagittal, and somewhat toward the left side of the body, and the dorsal margin lies toward the top of the page. Thus it appears that in P. gemellipara the migration of cells occurs as freely in the dorsal as in the lateral and ventral portions of the embryo. In the later stages, however, as Hallez pointed out, there are comparatively few nuclei on the dorsal side of the entoderm (fig. 37).

There are two types of nuclei in this sub-epithelial layer, an external row of small rather deeply stained nuclei which usually lie against the inner surface of the ectoderm cells, and an internal series of larger and paler nuclei, some of which are slightly irregular. The second type is more numerous on the lateral and ventral surfaces. The embryo at the left was tangentially sectioned, thus showing the more lateral nuclei.

Passing to the more advanced stage shown in the sagittal section in figure 37, where the brain and pharynx have become differentiated, it is found that certain of the cells containing the smaller nuclei have become spindle-shaped. The cytoplasm has formed a process at each end. These spindle cells are still more conspicuous in figures 27 and 36.

Bresslau ('04) describes similar cells at a corresponding period in the development of Mesostomum as representing the early stages in the differentiation of the dorso-ventral muscle fibers. The number observed just beneath the ectoderm of Paravortex rather suggests that from these cells arise the circular and longitudinal muscle fibers which soon become so conspicuous in this position (fig. 40). These sub-epithelial muscle cells of the adult Rhabdocoeles have been observed by many investigators. The figures in Bronn's "Klassen und Ordnungen" show the remarkable extent to which they become fibrillated.

In Paravortex as in most of the Rhabdocoeles, a muscle net is developed just beneath the epidermis. Figures 40 and 41 show the outer layer of circular and inner layer of longitudinal fibers in the adult condition. Since the figures in the various works which have been accessible never show nuclei in connection with the fibers of this muscle net, a vigorous attempt was made to find the cells from which these fibers arise in the embryo of Paravortex gemellipara. Of interest in this regard is the cell shown in figure 39, for it is possible to trace several long delicate fibers outward from the cytoplasm surrounding its nucleus. The latter lie close beneath the external epithelial cells in the position later occupied by the muscle net. Coe ('99) traced such a development of the muscle fibers in the Nemerteans Micrura caeca and Cerebratulus marginatus.

If this interpretation of the origin of the muscle fibers be correct, then a further question presents itself. Do these fibers later become independent or do they maintain a connection with such a cell body as is seen in figure 39? It is difficult to conceive, judging from the parallel arrangement of muscle strands in the adult, that the fibers are still in connection with these cell bodies. The latter, moreover, cannot be detected in the adult tissue. The conclusion is, therefore, that either the fibers become separated from the nuclei, or that the latter by repeated division are so reduced in size that they escape observation.

e. Sex organs and vitellaria. Not until after the young leave the mother's body does the true differentiation of the sex organs commence. At the time the young worm breaks out of the capsule and enters the surrounding mesenchyme the anlagen of the two hermaphroditic glands are only loose masses of cells, one on either side just lateral and posterior to the pharynx. In one animal which had forced its way into the mother's intestine a few cells of these paired anlagen were undergoing mitosis, while those of the neighboring tissues were entirely without mitotic figures (fig. 49).

Just previous to this stage of development occasional large cells make their appearance in the ventral region of the body between the entoderm and the layer of muscle fibers. These cells are represented in figures 37 and 43 as having larger, clear nuclei and a considerable amount of darkly stained cytoplasm. The color of the latter in iron-haematoxylin preparations is a bluish gray.

It is believed that these large cells later develop into the yolk cells of the vitellaria. The grounds for this conclusion will be brought forward in the section on post-embryonic development. But their behavior while the young worm is still enclosed in the capsule may be traced somewhat farther. Since they appear first in the region close behind the sex organ anlagen it is probable that they arise, either directly from them or from cells which have had a similar ancestry. It is known of other Turbellaria that the vitellaria become differentiated from the same mass with the ovaries and the testes (Hallez '09). So in Paravortex gemellipara, which at the height of its reproductive activity is characterized by the presence of remarkably extensive vitelline glands, the general rule holds concerning their origin; but in point of time their branching off from the common sex organ mass seems unusual.

f. Cilia. The epithelial cells become furnished with cilia shortly after the stage seen in figure 34. They are already present in figure 35, but of their development nothing has been observed beyond the fact that, although they are as long when first noted as in older worms, they are not nearly so numerous as on the adult epithelium. The embryos at this stage are able to move about in the capsules. The ciliary action can easily be observed through the maternal tissues when the living worm is studied.

g. Embryonic digestion and early development of the intestine. In an earlier section of the paper it was explained that the gastrulating embryo, like those of all Rhabdocoeles, enclosed within itself the free yolk material which had previously entered the capsule as a constituent of the vitelline cells. The greater portion was absorbed by primary entoderm cells which were in turn ingested by amoeboid secondary entoderm elements. The ectoderm grew backward over the entoderm and at the same time absorbed the remainder of the vitellarial yolk. Figure 23 was pointed to as illustrating the appearance of the embryo at the close of gastrulation. By far the greater portion of the embryo is occupied by the yolk-laden cells.

The fate of this yolk now remains to be described. In the stage represented by figure 23 little change has occured in the nutritive material. It is of the same nature as the free yolk seen in figure 21, with the exception that the flakes and globules are less closely packed in the cytoplasm of their containing cells. There is observable, however, a tendency of these particles to flow together into larger globules, a process which is more rapid in the ectoderm cells. Thus in the two epithelium cells at the top of figure 23 several such spheres are conspicuous.

In an older embryo (fig. 34) this sphere formation has advanced considerably. The spherules of nutritive substance combine again and again until the major portion is included in one or two immense globules. That the yolk is undergoing a transformation is indicated by the appearance of darker spherules suspended in the large masses. These smaller elements stain dark brown or black in iron-haematoxylin preparations such as figure 34 represents.

In figure 25 is illustrated the appearance of the ectodermic nutritive material in the living embryo after treatment with the neutral red stain. The latter has a more rapid effect upon the larger masses than upon the smaller. After immersion for a very few minutes in a dilute neutral red solution it is impossible to see through the posterior ectoderm; all is a mass of deeply stained red spheres.

Returning to figure 34 it is seen that clear regions have appeared in the interior of each entodermic yolk cell. These regions in the iron-haematoxylin-eosin preparations are perfectly colorless, but in those treated with Ehrlich's haematoxylin and eosin after fixation with corrosive acetic, they take the eosin color (figs. 27, 36). Thus it is evident that these lighter portions are filled with a plasma. To how great an extent the separation of the yolk-filled contents into two zones is due to the action of reagents has not been determined. It is interesting to note in this connection that most of the spherical yolk-mass enclosed in the embryo in figure 42, an Ehrlich-stained specimen, is homogeneously filled with yolk-granules such as originally entered the cells. Still this embryo, as indicated by the presence of the cilia, is more advanced in development than the one illustrated in figure 34.

One entoderm cell, that at the upper left in figure 42 demands attention. The yolk material which it contains has entirely assumed the fluid nature seen in central regions of these cells (fig. 36). Now it has been explained that the Flemming-iron-haematoxylin-eosin method leaves these regions entirely colorless. Hence the cell now under consideration, if stained by this latter method, would have apparently contained a vacuole in the position here occupied by the yolk mass. Exactly such a condition is exhibited in figure 37. In this embryo there appear what at first were regarded as vacuoles within a mesenchymatous network; they are now rather to be interpreted as the transformed yolk material accumulated within the entoderm cells as immense globules which, following the corrosive-acetic Ehrlich's haematoxylin-eosin method, stain a rich yellow. It has been pointed out that by this method also the nuclei, cytoplasm and membranes of the entoderm cells containing these nutritive masses are much more clearly stained than by the iron-haematoxylin.

Another feature to be noted in the entodermic yolk masses in figure 42 is that the mitochondrial mass is again discernible. This body was traced from its origin within the oocyte through cleavage to its division and enclosure in the primary entoderm cells. During the early history of the mass the Ehrlich stain was unfavorable to its demonstration, since the cytoplasm of the cell retained so deep a stain that the mitochondrial mass was obscured. But in the late stage shown in figure 42 the cytoplasm of the original cell has broken down and the granular mass of mitochondria again comes into prominence. The centrosome with which it was earlier associated may still be detected, although less easily than during the period of its activity.

A certain fact favors the view that a process of disintegration has attacked the mitochondrial substance. For whereas, up to the time when the entoderm cells had absorbed a considerable amount of yolk, the body appeared conspicuously in iron-haematoxylin material, it has been found impossible to detect it in such preparations at later stages of development. This peculiarity, taken in connection with the ability of the Ehrlich stain to bring it out subsequently, points to the conclusion that the mitochondrial substance has become chemically changed. Furthermore, no trace of it is to be observed after the yolk globules have been transformed into such a fluid mass as noted in the upper entoderm cell of figure 42. It would be too extreme to surmise that the mitochondrial mass represents a substance laid down in the oocyte for the purpose of acting as a digestive agent after the yolk has been taken inside the embryo. Such an interpretation would have to regard the substance as a potential ferment, which, before it could act upon the yolk, must undergo a transformation which renders it invisible.

Against the view that this mitochondrial mass is functional at this stage is the evidence furnished by the ectoderm. Here a simultaneous yolk-digestion has been in progress where none of the mitochondrial substance entered. In figures 35 and 37 each posterior epithelial cell contains one or more of the large fluid spheres. In appearance and reaction to the Ehrlich's haematoxylin-eosin stain, these masses are closely similar to those in the entoderm. As we have seen, their origin also is the same. In the iron haematoxylin preparations, however, the ectodermic material stains brownish while the entodermic masses are colorless. It is probable that both substances are lipoids of some sort.

After the young worm has left the capsule and is prepared to take food through the mouth the reserve material present up to this time in the ectoderm rapidly disappears. The process is apparently one of gradual absorption and assimilation. In one preparation a cell just beneath and closely applied to the ectoderm contained two small globules, of the same consistency and color as the food material in the entoderm. It is possible that through the agency of mesenchymatous cells the ectodermic nutriment is passed on to the tissues of the embryo. On the other hand this cell may have belonged to the entoderm, its contents representing the last stage in the digestion of the entodermic yolk.

During the absorption of the reserve food, the ectoderm, or external epithelium of the young worm, has undergone vacuolization; the cytoplasm of each cell now forms trabeculae between irregular clear regions (fig. 37). This condition is at first more noticeable at the anterior end of the body where less yolk is deposited, but with the absorption of the latter posteriorly the whole ectoderm takes on this appearance. This vacuolization causes a marked increase in the depth of the cells.

h. Formation of the intestine. According to Bresslau ('04) the intestinal epithelium is formed in Mesostomum by previously undifferentiated embryonic cells which unite first in the region just posterior to the esophagus. Gradually this sheet of cells, by the addition of others, extends posteriorly about a cavity which arises as a splitting of the tissues in the region of the fast shrinking yolk cells. At the same time the intestinal cells become vacuolated.

Hallez ('09) asserted that the cells which enter into the formation of the intestine of Paravortex cardii are of the same nature as those which give rise to the mesenchyme. He considers their immediate predecessors to be large cells lying at the posterior end of the body. 'These proliferate at once cells which are added to the intestinal 'syncytium,' and others which constitute the mesenchyme between the intestine and body wall. He observed that a lumen appears first in the region just behind the oesophagus, and gradually, with the absorption of the vitelline spheres, extends posteriorly.

From certain observations of the conditions under which the intestine arises in Paravortex gemellipara there is some doubt that it originates in the manner described by Hallez for P. cardii. It seems rather that the large posterior cells, as noted in the preceding section on the sex organ and vitellarium anlagen, ultimately differentiate to form the vitelline cells. Their development will be considered later.

In P. gemellipara the intestine is organised from cells which, at the stage illustrated by figures 35 and 36, are proliferated in the region just posterior to the oesophagus. Two of these in figure 26 were pointed out above while the pharynx was being considered. It is believed that they are derivatives of the large clear cells designated as *ent. 2* in figure 23.

With the rapid absorption of the yolk inside the secondary entoderm cells, the latter become enclosed with the epithelial cells which, arising in the manner above described, migrate backward about and between them. In this way a loose rod of cells comes to occupy the whole region behind the pharynx. As Hallez observed, the intestinal lumen first appears at the anterior end of the rod, and gradually extends posteriorly. As long as the young worm remains in the capsule there is no communication with the outside through the pharynx and mouth. It is probable, however, that it is potentially present, requiring only the muscular activity of the pharynx to open the lumen. The young animals, when liberated from the mother by pressure upon the cover-slip, exhibit contraction and expansion of the pharyngeal apparatus, a feature which indicates that, as soon as they are in a position to obtain food from without, they are provided with a lumen from mouth to intestine.

i. Glands. Shortly before the young worm leaves the capsule from two to four elongated oval or spindle-shaped bodies appear between the brain and the vertex of the anterior end of the body (fig. 45). In iron-haematoxylin preparations these masses are grayish in color, while Ehrlich's stain imparts to them a deep red. In animals which have entered the tissues of the mother these gray bodies communicate with the exterior (fig. 46) by slender processes; they are now recognizable as the cephalic glands. To these has been attributed the function of secreting a slime which lubricates the animal's path.

Numerous other spindle-shaped cells distributed through the sub-epithelial tissue are evidently single-celled glands which have to do with the secretion of mucous.

9. *Post-embryonic development*

In order that the further development of the sex organs, intestine, mesenchyme and glands could be studied, an attempt was made to keep alive in small aquaria young worms which had been removed from adults, into whose parenchyma they had already become freed. No success was met with; all died inside of seventy-two hours.

Therefore several young of the smallest size obtainable from the mussels by ordinary methods were sectioned in the hope that some of the organs would be found in an undeveloped state. While this trial gave some interesting results, it is desirable that a study be made of specimens younger than these and at the same time older than those secured at the time of birth.

The following observations are submitted as being of interest in this connection.

a. The definitive intestine. Hallez describes and figures the adult intestine of Paravortex cardii as consisting of a veritable syncytium, a mass of cytoplasm containing large vacuoles and scattered nuclei. The intestinal wall of P. gemellipara, however, consists of elements which are distinct except at their extreme bases. Figure 47 was drawn from a worm which measured 0.30 mm. in length. The intestine, having already assumed the adult condition, is seen to consist of large cells which extend from the outer surface of the organ inward to its lumen. Their bases are constricted and narrowed, while the distal ends assume a rounded form where they extend into the intestinal cavity. Numerous vacuoles, some of them containing food materials, are distributed throughout the cytoplasm. Most of the nuclei are located in the narrowed ends of the cells. Figures 8 and 9 exhibit a similar appearance of the intestine of old worms. A like structure of the turbellarian digestive tube is figured in such works as von Graff's Monograph and Bronn's "Klassen und Ordnungen,"

b. Mesenchyme. According to Hallez's ('09) account of P. cardii, the mesenchyme receives elements, both from the undifferentiated cell mass at the anterior end of the body and from the large cells which become grouped at the posterior extremity.

Bresslau ('04) also derived the mesenchyme from the undifferentiated cells.

The origin of this tissue in P. gemellipara agrees with that described by these investigators except that there is no evidence of a contributory action on the part of the large posterior cells; it is all derived from the undifferentiated cell mass, from which cells migrate posteriorly between the intestine and body wall. The mesenchyme of the free-swimming young worm from which figure 47 was drawn already existed in the condition which is characteristic of the adult. The cells are large and loosely arranged with indistinct and irregular boundaries.

c. Ovaries and testes. The youngest stage in the development of the sex glands of P. gemellipara in which they can be recognized is shown in figure 47. Although the testis lies close to the anterior end of the ovary, the two glands are already separate. No epithelium, however, has been formed about them. Since this specimen had been stained with Ehrlich's haematoxylin, and since the section was so thick that the entire testis and nearly all of the ovary are included, the minute condition of the chromatin cannot be determined. The small dimensions of the nuclei in comparison with those observed in the adult ovary indicate that all the cells in the female gonad, except possibly the most posterior, are oogonia.

An important feature in this specimen is that the vitellaria have become conspicuous, particularly in the posterior half of the body. No connection between these glands and the ovaries can be traced in this series of sections.

The next instructive stage which my material presented is shown in figure 50, a sagittal section through one testis, the seminal vesicle, atrium, shell-gland and the tissue which is to form the antrum. In the testis the spermatogonia and spermatocytes can easily be distinguished. Hallez described and figured a similar appearance for the young testis of P. cardii. One feature of which, however, he failed to give an account is that the gland becomes provided with a flat epithelium. The latter is derived from the mass of cells lying below the mid-ventral side of the intestine. From this same mass the accessory reproductive organs are also being differentiated. It is evident that the seminal vesicle arises from the anterior portion of the mass; only a small number of cells at the extreme anterior end go to form the vasa deferentia and the external epithelium of the testis.

In the region ventral and posterior to the seminal vesicle the atrium has differentiated simultaneously. Like those of the former, its walls are composed of two layers of cells, an inner cubical epithelium and an outer sheet of flattened cells. As yet no connection of its lumen with the outside of the body has been effected. Indeed, the cells are not even in close contact with the body wall.

From the posterior surface of the atrium a dense mass of cells leads a short distance dorsally. The antrum femininum will arise later in this position. On the ventral surface of the mass is a group of cells whose rounded posterior ends have begun to reach backward behind the atrium. These cells enlarge to form the single-celled shell-glands.

Of the oviducts nothing is as yet present but a loose string of undifferentiated cells which lead from the antrum anlage posteriorly and dorsally to the ventral surface of the ovary. It was impossible to distinguish about the latter such an epithelium as was described for the testis, nor is it evident in later stages.

The ovaries in the worm from which figure 50 was drawn are well advanced. The older oocytes have undergone a considerable growth.

Figure 51, from a somewhat larger worm, is presented to show clearly the strand-like structure of the testis and the point at which the vas deferens enters the seminal vesicle. The oogonia occupy the anterior region of the testis. These, by growth, give rise to the large primary spermatocytes lying on the ventral and dorsal sides; while in the center of the posterior half a row of smaller, densely stained cells are regarded as secondary spermatocytes. No spermatids were distinguished with certainty, but mature spermatozoa are conspicuous in the anterior central region whence they can be traced dorsally between the cell strands to the upper surface of the testis. Others lie just beneath the external epithelium and in the vas deferens, while many have already entered the seminal vesicle. Thus it is evident that Paravortex is protandrous to the extent that spermatozoa are produced before the female reproductive tract has become differentiated, for no lumen has appeared farther backward than the atrium.

d. Vitellaria. In the section wherein the earliest anlagen of the sex organs were described it was noted that several large cells with an unusual quantity of darkly staining cytoplasm and conspicuous nuclei were observed to arise in the region of the gonads and to migrate posteriorly between entoderm and ectoderm (fig. 43). While these cells became distributed on all sides of the latter, a marked accumulation occurs at the extreme posterior end (fig. 44). Hallez was of the opinion that these cells in P. cardii contributed largely to the formation of the intestine, but also to the mesenchyme and vitellaria. There is no evidence that they give rise in P. gemellipara to any other organ than the vitellaria. These cells are identical in appearance with the young cells which lie at the extreme tips of the vitelline strands in the adult. Figure 44 shows at once the embryonic cells and, just outside the capsule, several of the young vitelline cells in the mother's tissue.

By the time the young worm has attained the stage represented in figure 47 strands of cells similar to those of the adult have grown forward from the posterior mass; at the same time the scattered cells of the same type have divided to form groups here and there through the mesenchyme of the posterior region. At x two of them are undergoing mitotic division; similar figures have been observed at the tips of the adult vitellarial strands. As yet very few have appeared in proximity with the ovaries. Hence there seems to be no evidence that in P. gemellipara the vitelline glands develop, even in part, from the hermaphroditic gland as it appears at this stage; these organs lack all connection with any other densely stained tissue. In the reconstruction (fig. 52) from an older worm, however, the vitellaria have formed a contact with the posterior ends of the ovaries. The various groups of yolk cells have anastomosed to form an extensive gland.

V. DISCUSSION

In the preceding account of the development of Paravortex gemellipara little attention has been paid to the interpretation given by Hallez ('09) of the processes of yolk absorption and the origin of the ectoderm in P. cardii.

He maintains that, while the morula is being formed by the cleavage of the egg, the membranes of the yolk-cells included in the capsule disintegrate, but that a part of their nuclei remain prominent and active. Hallez finds these nuclei shortly afterward lying in a clear plasma-like portion of the yolk which has become separated from an eosin-staining constituent and has assumed a peripheral position outside of and between the embryos. On the other hand the 'eosinophile' portion of the yolk separates, he believes, because its chemical and physical properties are different from those of the cytoplasmic remainder. As these properties become more pronounced, the central yolk material splits off in the form of great drops in whose interior he finds a variable number of vacuoles. He has figured several nuclei, which are assumed to have been set free by the disintegrating yolk cells, lying in the outer clear zone; others have migrated inward between the embryos. As pointed out earlier in this paper, he found that one of these nuclei became associated with each of the 'balles vitelline.'

Even more remarkable is Hallez's account of the developmental phenomena which immediately follow. Into each vitelline sphere one of these migratory nuclei penetrates, together with a small quantity of the cytoplasm which also was previously a constituent of the vitellarium cells. At the same time the material of these yolk spheres assumes a granular appearance.

A little later these nuclei, he states, emigrate with their associated cytoplasm from the 'balles vitellines.' Part of these amoeboid cells are asserted then to mingle with the envelope of plasma outside the embryo so as to form a 'syncytium' from which a primary ectoderm soon differentiates. Other such cells delaminate from the yolk spheres as additions to the ectoderm. Into the formation of the latter now enter, as Hallez believes, the remaining vitellarial nuclei which did not previously penetrate the yolk spheres. Hallez observed also that the true embryonic cells contribute to the development of the ectoderm. This ectoderm is retained permanently. The remaining 'migratory cells' form another 'syncytium' about the vitelline spheres which they have just left, this constituting, in Hallez's opinion, the primary intestine.

The ectoderm on the apposed surfaces of the two embryos is formed by a delamination through the center of the yolk-cell derived plasma with its migratory nuclei. Hallez discerned but few cell membranes.

From a study of his figures and descriptions it seems possible that owing to a failure to distinguish the extremely delicate outlines in the embryonic structures he has been led to the above interpretation. I believe that further study may show that the processes of early development in Paravortex cardii are very nearly the same as those which I have found in Paravortex gemellipara.

The derivation of the ectoderm and entoderm as observed in the development of P. gemellipara agrees essentially with that determined by Bresslau for the several species of Rhabdocoeles which he studied. A variation in the manner of yolk absorption, however, occurs in Paravortex. Whereas he found the yolk cells either to retain their envelopes, as in Mesostomum ehrenbergi, or to be enclosed by the ectoderm in the form of numerous yolk spheres resulting from the breaking down of the vitelline cells, in P. gemellipara the yolk granules are first absorbed by the primary entoderm cells, and these are in turn ingested by the secondary entoderm. The first step of the process is similar to that known to occur in the chick, where the intestinal entoderm absorbs the yolk particles.

That the yolk-absorptive function of these entoderm elements is their chief capacity is further indicated in a freak embryo shown in figure 53. This specimen, beside lacking the yolk-filled masses so characteristic of normal embryos, exhibits a remarkable distribution of the undifferentiated tissue. The latter has proliferated at a normal rate, so that following its posterior migration it has become arranged many rows thick between the ectoderm and a few large clear cells in the center. The latter are believed to belong to the primary entoderm which ordinarily absorbs the yolk; in this case it is probable that no food was enclosed with the egg in the capsule.

The behavior of the entoderm cells in P. gemellipara is comparable to that observed in 1884 by Lang in the Polyclad Discocoelis. Since the egg of this worm contains a rich supply of yolk material, none is added in the form of vitellarial cells. During segmentation an upper and a lower quartet of entoderm cells arise whose derivatives later form the wall of the alimentary canal. These entoderm cells have a more or less amoeboid character, sending out protoplasmic processes over the yolk spheres. The latter were previously split off from the yolk-filled middle entoderm which takes no part in the formation of any organ.

In 1907 Surface described the development of the alimentary canal in Planocera inquilina. He found that practically all of its tissue arises from two large entoblast cells. By division they form two sorts. One group containing the yolk is pushed into the interior of the embryo where the cells break down and the yolk granules flow together into a fluid mass of large spheres. Meanwhile the lower entoblast cells divide rapidly, thus giving rise to a large group of cells. A cleft, the first indication of the intestinal lumen, now occurs in this entodermic mass, and cell outlines become distinct about the nuclei. The important feature to be noted in relation to the behavior of the entoderm of P. gemellipara is that Surface observed these cells to become amoeboid and to spread out on the surface of the large yolk spheres which are thus absorbed.

Now it is only a short step from the condition described by Surface in a Polyclad to that which I have observed in the Rhabdocoele, Paravortex. Here two entoderm elements—the large macromeres of text figure 14 -give rise by division to the primary and secondary entoderm cells. The former, since they contain no yolk of their own, absorb it from the vitellarial substance. Like the upper yolk cells in Planocera, their nuclei degenerate and their membranes break down. But this latter step occurs only after they have been ingested by the secondary entoderm cells, which thus resemble in behavior the cells of the alimentary canal in Planocera.

Hallez states that in P. cardii no membrane is formed over the surface of the yolk mass by a part of the vitellarial cells. P. gemellipara resembles P. cardii in this respect; no shell-membrane arises. But Hallez thought he found a similarity between the behavior of the yolk-cell nuclei of Paravortex and Mesostomum in that certain of these nuclei in the former played an important part in the differentiation of the definitive ectoderm and primary intestine; according to Bresslau, in Mesostomum ehrenbergi, the peripheral yolk cells form a shell membrane over the surface of the remaining yolk. In Paravortex gemellipara, however, every cell which enters into the structure of any organ is a derivative of the egg. The vitellarial yolk-cell nuclei and membranes disintegrate, leaving only the yolk as food; the nuclei never contribute to the formation of living tissue.

Of the mitochondrial mass little remains to be said. Its history from the first appearance in the young oocyte was traced until it finally disappeared in the entoderm of the young worm. The suggestion was tentatively made that the body might be or might produce an enzyme which has to do with the transformation of the yolk. But a similar transformation took place in the ectoderm, a tissue which received none of the mitochondrial mass. It therefore appears that the literature concerning the 'Dotterkern' has received another contribution, but without further explanation of its action. Truly suggestive, however, is the behavior of the body.

VI. SUMMARY

1. The origin of two or more embryos found in the capsules of Paravortex gemellipara is similar to their origin in other capsule-forming Rhabdocoeles; that is, by the enclosure of two or more eggs and about one hundred yolk cells in a shell secreted by the shell glands.

2. The mitochondrial mass (yolk-nucleus) was traced from its origin in the young oocyte through cleavage to its final resting

place in the several primary entoderm cells. Its function probably has to do with the absorption and digestion by these cells of the vitelline yolk. The behavior of the mitochondrial mass is different from that observed in other animals in that, whereas in most forms it either disappears in the egg stage or, if remaining through cleavage, is restricted to one cell, in P. gemellipara several cells ultimately receive a portion of the original substance.

3. Three germinal regions are recognizable in the blastula, the mes-ectoderm, primary entoderm and secondary entoderm. These germinal regions do not seem to have been previously recorded in Turbellaria.

4. The vitellarial yolk-cell membranes and nuclei degenerate after deposition in the capsule; no temporary epithelium is formed by them outside the yolk mass.

5. The primary entoderm cells which lie as a cap at the posterior end of the embryo, absorb a large part of the vitellarial yolk. When replete their nuclei immediately degenerate.

6. These cells are then grasped by the amoeboid secondary entoderm cells. After gastrulation the latter completely engulf and digest the former.

7. During gastrulation the ectoderm cells absorb the free vitellarial yolk left over after the action of the primary entoderm.

8. The development of the eyes embraces two phases. First the accessory pigment cell arises as a large clear vesicle very early in the differentiation of the embryo, and becomes gradually filled with melanin granules which crowd the nucleus to one side. Secondly the essential organ, the sight cell, develops externally to the pigment cell, into an invagination of which it pushes, and sends a nerve fiber backward into the anterior horn of the brain.

9. The sub-dermal muscle fibers develop in this Rhabdocoele, as in Nemerteans, by the differentiation of mes-ectodermic cells from which long slender fibers grow outward beneath the external epithelium.

10. The intestine consists of large distinct cells whose free ends extend into the lumen. It arises after birth by the inter-

calation between the yolk-laden primary entoderm cells of new cells derived from the secondary entodermic elements which during gastrulation remain in the region just posterior to the oesophagus.

11. The approximate time of appearance was noted for the subdermal glands and those of the alimentary tract; they are not present in the young worm at the time it leaves the mother, but have already developed in free-living specimens which have reached a length of 0.30 mm.

12. The cephalic glands appear in the embryo while it still lies in the capsule. There are three single-celled glands which develop in the mesenchyme between and anterior to the eyes. By the time the young worm is ready to leave the mother these glands have each become connected with the exterior through a fine process which penetrates the anterior body covering.

13. During very early embryonic life the vitellogenous cells migrate backward from the region of the gonads—just posterior to the pharynx. Most of them become grouped in the posterior end of the body, but a number come to rest at various points beneath the ectoderm in the posterior two thirds of the body. The vitellaria develop first from the large mass of cells between the intestine and the body wall and push gradually forward till they effect a union with the posterior end of the antrum. Meanwhile the isolated cells divide to form groups which soon anastomose with the main portions of the glands.

The testis and ovary of each side of the body become differentiated from a single gonad, the former arising from the anterior and the latter from the posterior end. In a 0.30 mm. embryo the two glands have become separated. Meanwhile a large group of cells has accumulated in the mid-ventral line between intestine and body wall. The anterior portion of this mass proliferates cells which surround the testis with a flat external epithelium and also form the vasa deferentia. The seminal vesicle arises in the next posterior portion of the mass by a rearrangement of cells about a lumen. It is composed of two layers of epithelia. At the same time the atrium commune develops in a similar manner. At this stage the antrum femininum and oviducts exist only potentially in the undifferentiated mass posterior to the atrium.

The shell glands develop below the antral mass before the lumen of the latter appears. At this stage the testis has become functional; one can easily distinguish spermatogonia at the anterior end, the primary and secondary spermatocytes, spermatids and mature spermatozoa. Many of the latter now appear in the thick-walled seminal vesicle. The genital pore does not open until later.

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PLATE 1

EXPLANATION OF FIGURES

1 Outline from life of P. gemellipara somewhat flattened under a cover slip. Several stages of embryonic development shown within capsules × 64.

2 Three animals natural size and five others × 5 in characteristic shapes and poses.

3 The entire animal viewed from the left side as a semi-transparent object. To show particularly the position of the reproductive organs and their relation to other organs. × 88.

4 Frontal section through the upper end of the antrum femininum, the ends of the vitellaria and the most mature oöcytes of each ovary. × 200.

5 A spermatozoon. × 606.

6 Youngest third of the ovary to show the oögonia, development of the nucleolus, and particularly the development of the mitochondrial mass. × 606.

7 Portion of an oöcyte to show the structure of the cytoplasm and mitochondrial mass. × 606.

ant.♂, antrum masculinum
ant., antrum femininum
at., atrium commune
br., brain
cap., capsule
cyt., cytoplasm
e., eye
gl.c., cephalic glands
gl.sh., shell glands
g.p., genital pore
int., intestine
m., mouth
mes., mesenchyme
mit., mitochondrial mass
nat.s., natural size
n.op., optic nerve
nuc., nucleus
nucl., nucleolus
o., ovary
ooc., oocyte
oog., oogonia
ph., pharynx musculature
s.v., vesiculum seminalis
t., testis
v.d., vas deferens
vest., vestibule
vit., vitellarium
y.c., yolk cell

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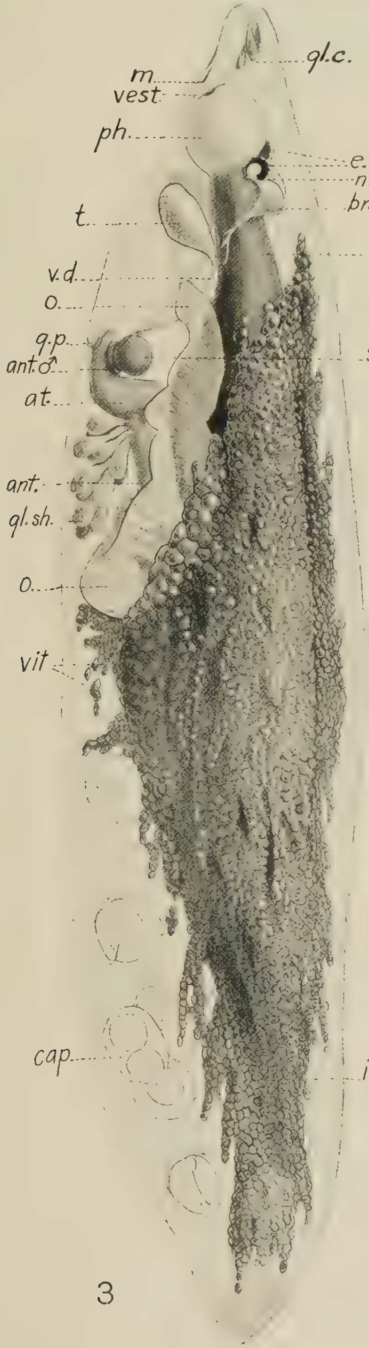
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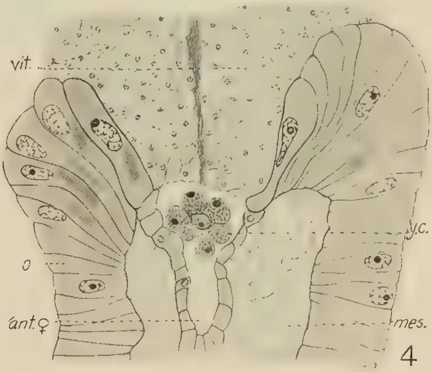
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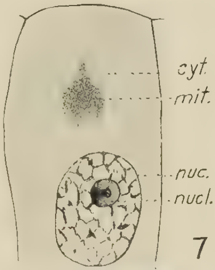
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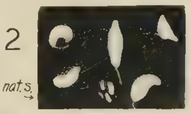
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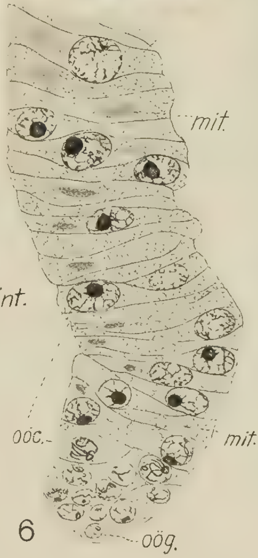
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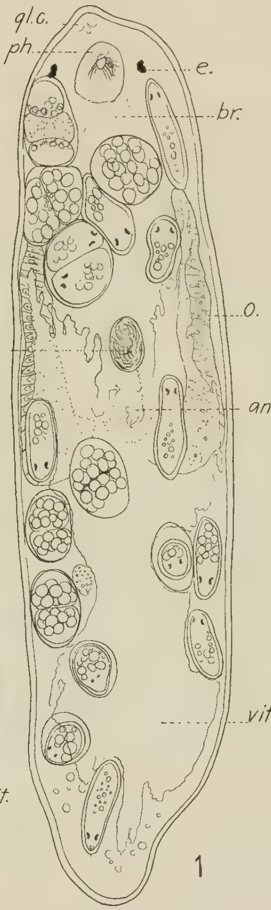
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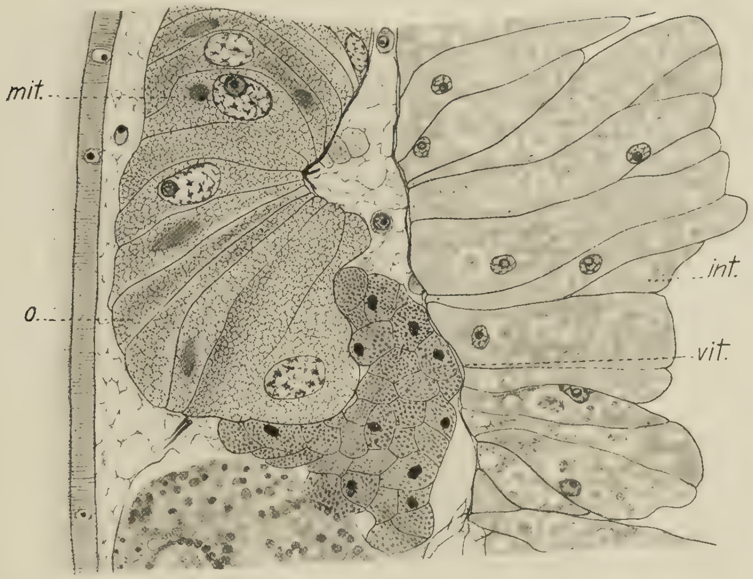
PLATE 2

EXPLANATION OF FIGURES

8 Frontal section through the posterior end of the ovary as it curves inward toward the oviduct. Note the definite membranes about the ova. × 270.

9 Oblique sagittal section showing the crucial stage in the formation of the capsule. Two ova are being surrounded together with yolk cells by the secretion of the shell glands. Numerous spermatozoa present about the eggs, and a mass of them in the antrum. × 270.

ant., antrum femininum
at., atrium commune
int., intestine
mit., mitochondrial mass
o., ovary
sh.gl., shell glands
sh.sec., secretion of shell glands
sp., spermatozoon
s.v.. vesiculum seminalis
vit., vitellarium
y., yolk



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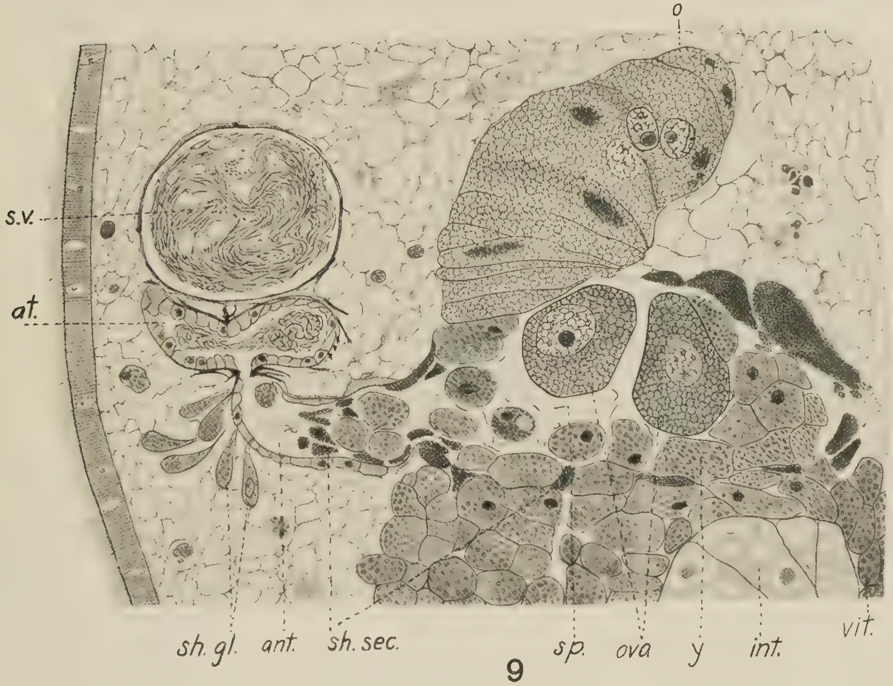

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PLATE 3

EXPLANATION OF FIGURES

10 Section of capsule showing first polar body at the left of the egg, and the male pronucleus inside. Several degenerating yolk cell nuclei and one degenerating spermatozoon in the yolk. × 730.

11 Another section of the same capsule in which the yolk cell membranes have not yet disintegrated. × 730.

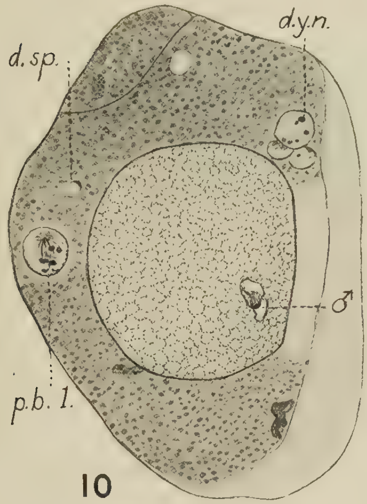
12 Drawing from life of a capsule freed from the mother by pressure, showing natural condition of yolk and mitochondrial mass, one egg and one embryo. × 520.

13 Two eggs of one capsule showing progressive steps in the cutting off of the first micromere. × 730.

14 Section through an embryo of about 72 cells. Three germinal regions are indicated, the primary entoderm (4 cells showing) at the left, mes-ectoderm at the right and secondary entoderm lying between. Yolk cell membranes and nuclei have entirely disintegrated. × 730.

15 Drawing from life of an embryo at the same stage as that in the preceding figure. × 730.

cap., capsule
cent., centrosome
d.sp., degenerating sperm
d.y.n., degenerating yolk cell nucleus
ent.1, primary entoderm
ent.2, secondary entoderm
inf., infiltration
mic.1, first micromere
mit., mitochondrial mass
p.b.1, first polar body
y., yolk
y.c., yolk cell
♂, male pronucleus



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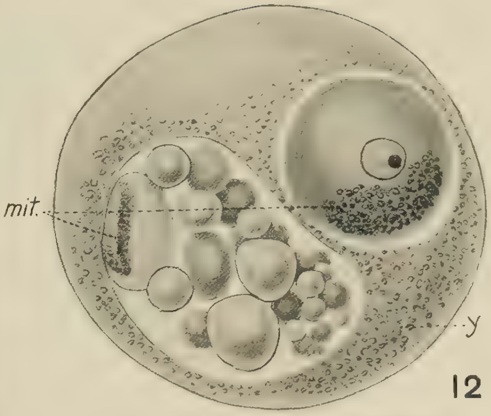

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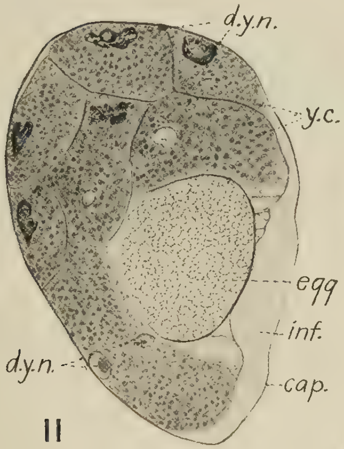

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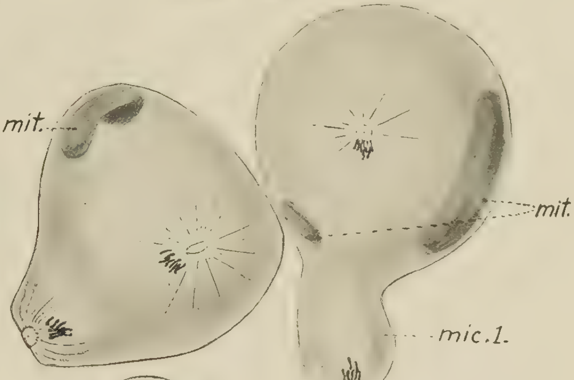

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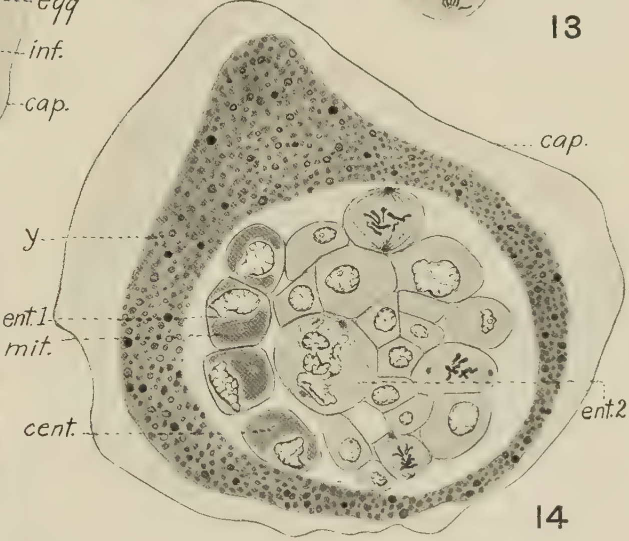

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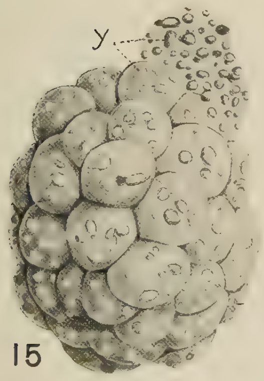

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PLATE 4

EXPLANATION OF FIGURES

16 Section through an embryo to show clearly the three germinal regions, mes-ectoderm, primary and secondary entoderm. × 730.

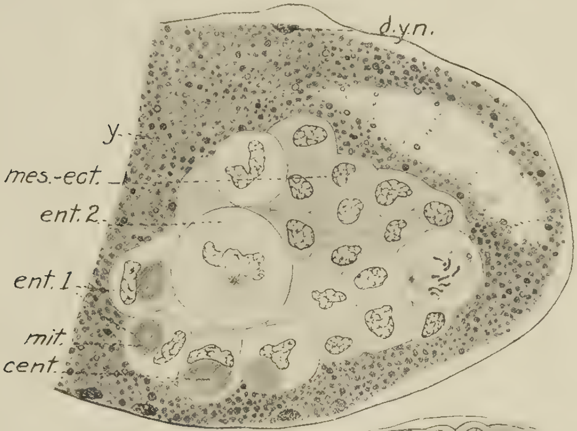
17 Section through a capsule of which the two embryos have begun to absorb yolk. This process is carried on by the secondary entoderm cells; each shows the mitochondrial mass and several yolk granules. × 730.

18 Section through part of a capsule in which the secondary entoderm cells have wandered to the posterior side of the primary entoderm. Most of the yolk has been absorbed by the entoderm. × 730.

19 Cell showing probable method of division of the mitochondrial mass. × 520.

20. Mitochondrial mass after its first division. Centrosome inside at the left, outside at the right. × 520.

cent., centrosome
d.y.n., degenerating yolk cell nucleus
ect., ectoderm
ent.1., primary entoderm
ent.2, secondary entoderm
mes-ect., mes-ectoderm
mit., mitochondrial mass
y., yolk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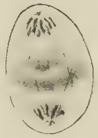

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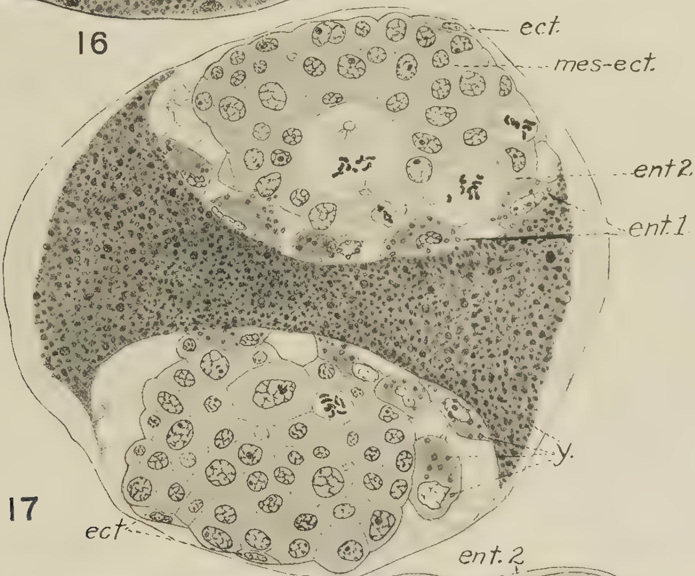
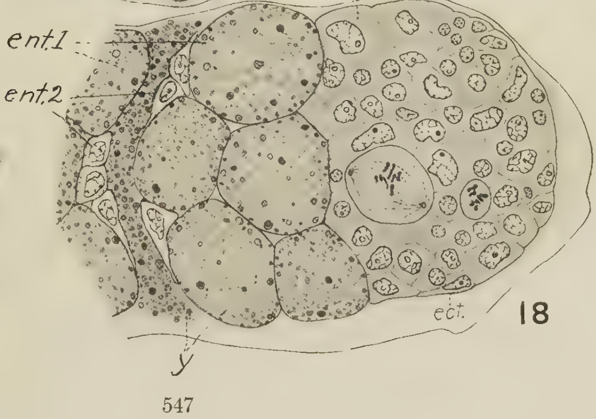

ect.
mes-ect.
ent 2.
ent. 1
y.
ect.

17


ent. 2
ent. 1
ent. 2
ect.
y

18

20

PLATE 5

EXPLANATION OF FIGURES

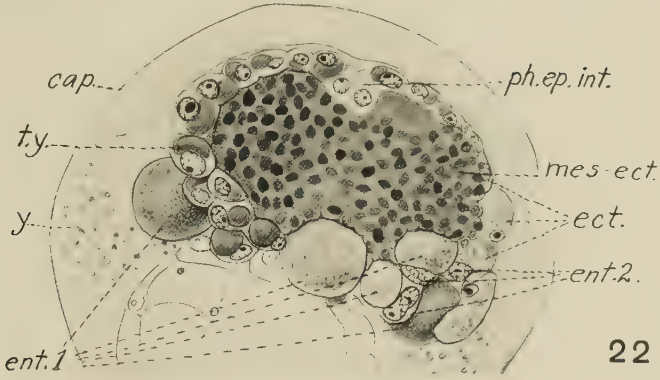
21 Section through two embryos of a capsule abundantly supplied with yolk Nuclei of the primary entoderm cells are undergoing degeneration while these cells are being engulfed by those of the secondary entoderm. At *ect.* the mes-ectodermic cells are becoming flattened to form the ectoderm. × 730.

22 Thick section of capsule in which the upper embryo is in the process of gastrulation. The ectoderm cells have nearly closed over the entoderm. The primary entoderm cells have been grasped by those of the secondary entoderm. At the right three ectoderm cells are absorbing free yolk left in the capsule after the entoderm had become replete. Ectoderm at the left contains yolk which has already been partially transformed. × 730.

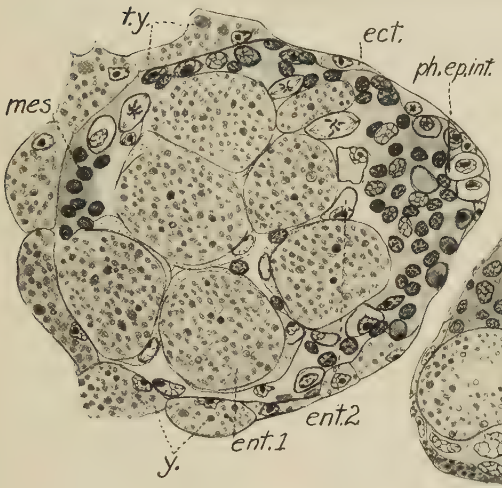
23 Oblique frontal section of an embryo just after gastrulation has been completed. Same features as in preceding figure. The ectodermic yolk beginning to be transformed (cf. the two cells at the top). The cells from which the intestine is mainly to be derived are shown (*ent. 2*) lying between the yolk-laden cells and the undifferentiated anterior cell mass. On the lower right several large nuclei with their cytoplasm are to be added to the ectoderm. × 730.

24 Oblique sagittal section of a slightly later stage in which the mesodermic cells have pushed backward between the posterior body wall and entoderm. Eye pigment cell just differentiated. × 730.

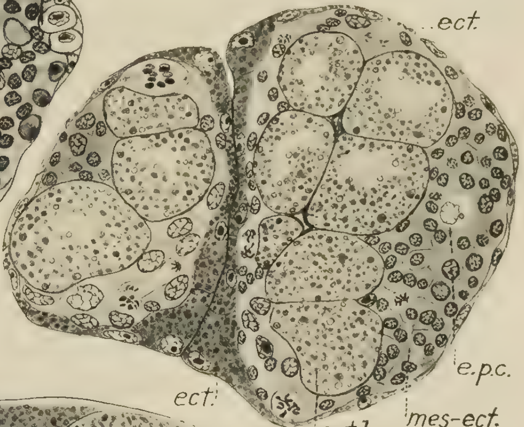
cap., capsule
ect., ectoderm
ent.1, primary entoderm
ent.2, secondary entoderm
e.p.c., eye pigment cell
mes., mesenchyme
mes-ect., mes-ectoderm
ph.ep.int., internal pharyngeal epithelium
t.y., transformed yolk
y., yolk


cap.
t.y.
y.
ent.1
ph.ep.int.
mes-ect.
ect.
ent.2.

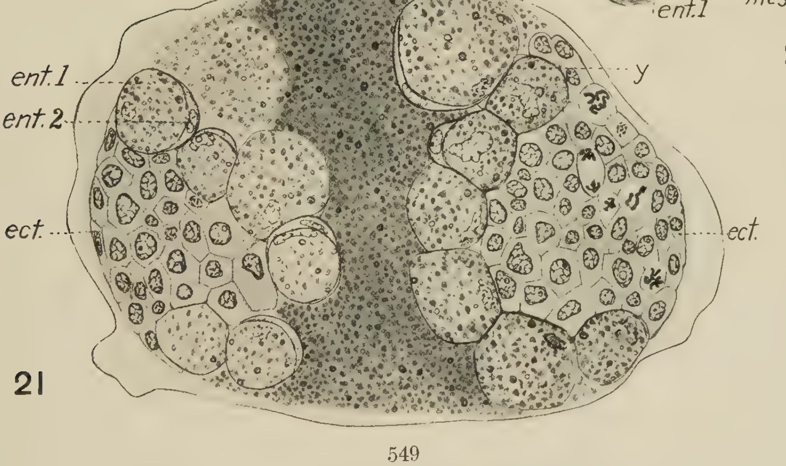
22


t.y.
ect.
ph.ep.int.
mes.
ent.2
ent.1
y.

23


ect.
e.p.c.
ect.
ent.1
mes-ect.

24


ent.1
ent.2
ect.
y
ect.

21

PLATE 6

EXPLANATION OF FIGURES

25 Drawing from life of an embryo at the stage seen in the preceding section (fig. 24). The globular nature of the free yolk is well shown. Water had infiltrated under observation between capsular membrane and yolk. Stained intra vitam with neutral red. × 730.

26 Frontal section of an embryo at same stage as that in preceding figure. Same features as in the latter and also the secondary entoderm cells (*ent. 2*). × 520.

27 Transverse section through pharynx and most anterior entoderm cells. Internal pharyngeal epithelium clearly illustrated in section, and the cells which are to form the pharynx musculature are grouped around this epithelial rod. × 520.

28 Frontal section of anterior end of an embryo to illustrate position of the two eye pigment cells. × 1016.

29 Oblique transverse section through brain and posterior nerve trunks. × 730.

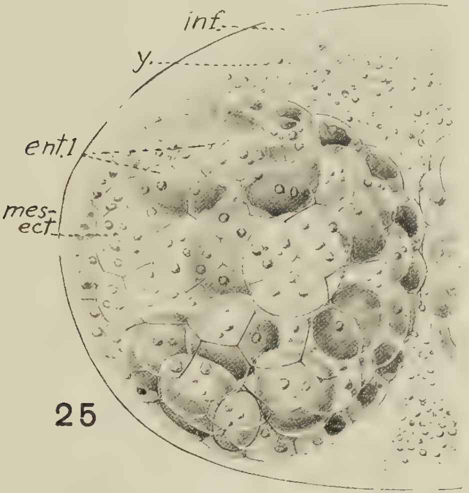
30 Series of drawings to show one type according to which the pigment granules of the eye appear one after another. × 530.

31 A second series to show another type of melanin deposition whereby several tiny spherules appear together and enlarge gradually. × 530.

32 Eye of an older embryo which had escaped from its capsule. Pigment cell has become invaginated by pressure of the ganglion cell (*g.c.*) from which nerve fibers extend into the brain. × 730.

33 Adult right eye seen from above showing the sight cell at the end of the optic nerve. × 730.

br., brain
ect., ectoderm
ent.1, primary entoderm
ent.2, secondary entoderm
e.p.c., eye pigment cell
esoph., esophagus
g.c., ganglion cells
inf., infiltration
m.emb., embryonic muscle cell
mes., mesenchyme
mes-ect., mes-ectoderm
n., nucleus
n.op., optic nerve
n.p., posterior nerve trunk
pct.s., punktsubstance
ph., pharynx musculature
ph.ep.int., internal pharyngeal epithelium
t.y., transformed yolk
vest.ep., epithelium of vestibule
y., yolk



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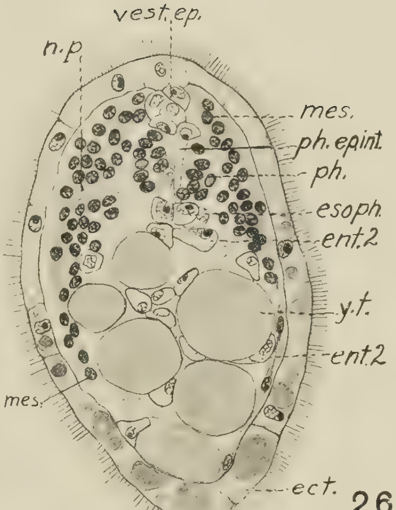

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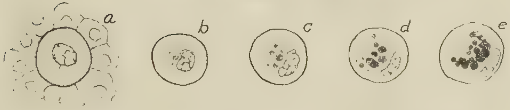

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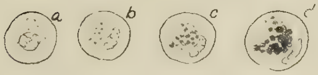

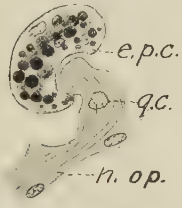

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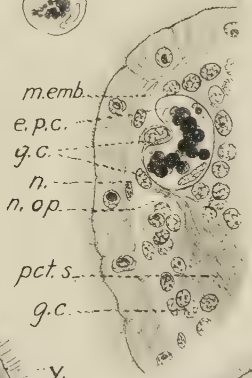

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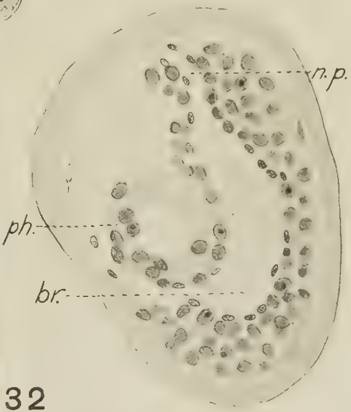

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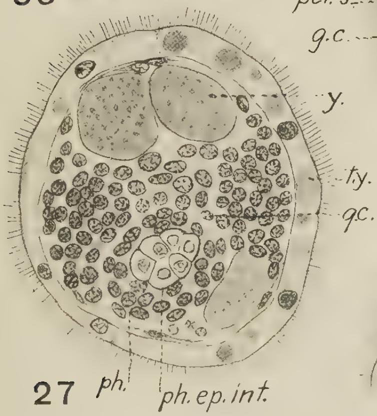

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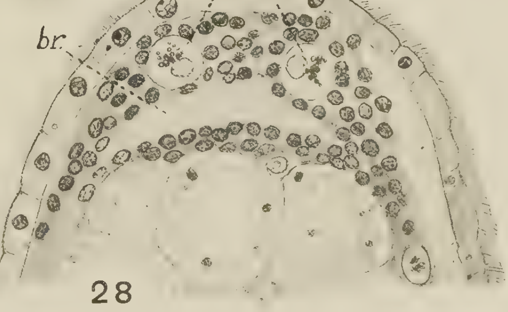

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PLATE 7

EXPLANATION OF FIGURES

34 Oblique sagittal section of an embryo somewhat more advanced than that in figure 33. Digestion of the yolk has begun; clear masses of transformed yolk occupy the center of each entoderm cell, while in the ectoderm the yolk has formed several irregular masses in each cell. The anterior ectoderm has become vacuolated. Between ectoderm and entoderm muscle cells are forming from mesodermic elements. Brain has differentiated and the internal epithelial rod has grown inward through the indistinct mass of pharyngeal muscle cells. Position of the mouth indicated at *m*. × 530.

35 Oblique sagittal section showing brain, eye pigment cell, entoderm and muscles. × 530.

36 Transverse section of same embryo as that shown in figure 27, but farther posteriorly, passing through the cells which later form the esophagus. × 530.

37 Oblique sagittal section of an older stage than that shown in figure 34. Entodermic yolk completely transformed (does not stain with iron haemotoxylin) while that in the ectoderm exists as large spheres. Pharynx distinct and early vitellogenous cells (*vit c.*) differentiated. Cilia have developed. × 730.

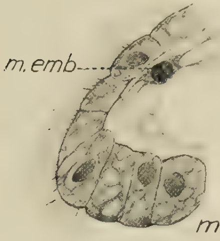
38 Transverse section of pharynx to show its musculature, and internal and external epithelia. × 730.

39 Tangential section through body wall of an embryo which had become freed into the mesenchyme of the mother. Muscle cell (*m. emb.*) from which extend fine muscle fibrils; the latter become the definitive sub-dermal fibers. × 1016.

40 Portion of a transverse section of the dorsal body wall showing outer circular and inner longitudinal sub-dermal muscle fibers. × 1016.

41 Adult sub-dermal muscle fibers. × 1016.

ect., ectoderm
ent.2, secondary entoderm
e.p.c., eye pigment cell
esoph., esophagus
g.c., ganglion cells
gon., gonad
m., mouth
m.emb., embryonic muscle cell
m.f.c., circular muscle fibers
m.f.l., longitudinal muscle fibers
pct.s., punktsubstance
ph., pharynx musculature
ph.ep.int., internal pharyngeal epithelium
ph.ep.ext., external pharyngeal epithelium
t.y., transformed yolk
vit.c., vitellogenous cells
y., yolk



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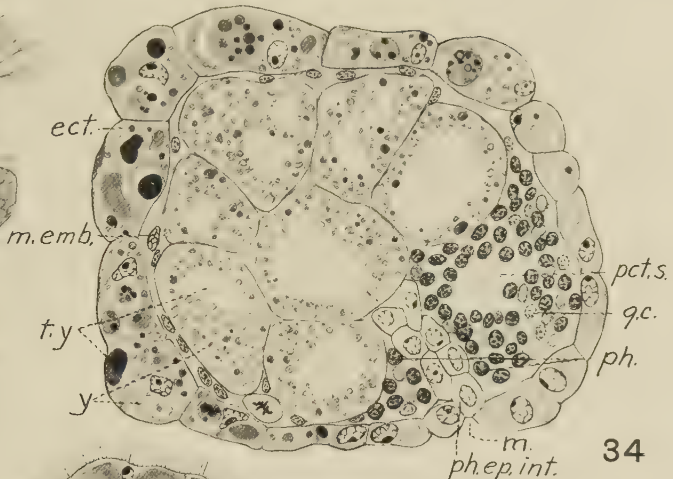

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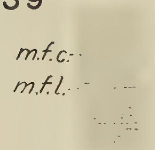

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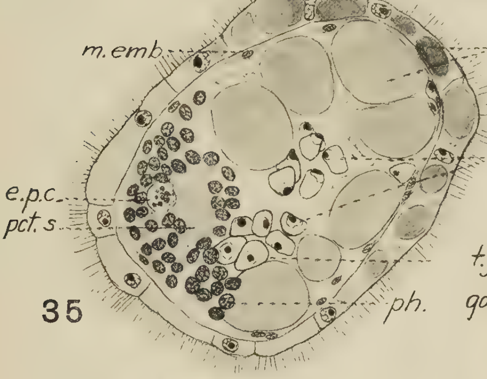

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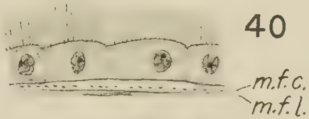

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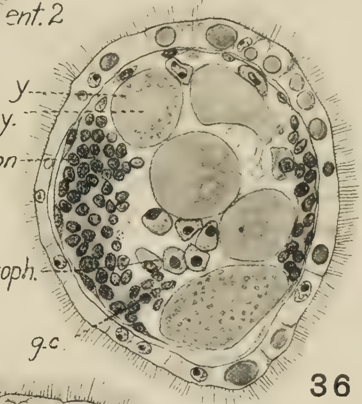

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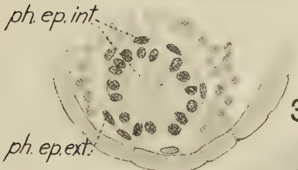

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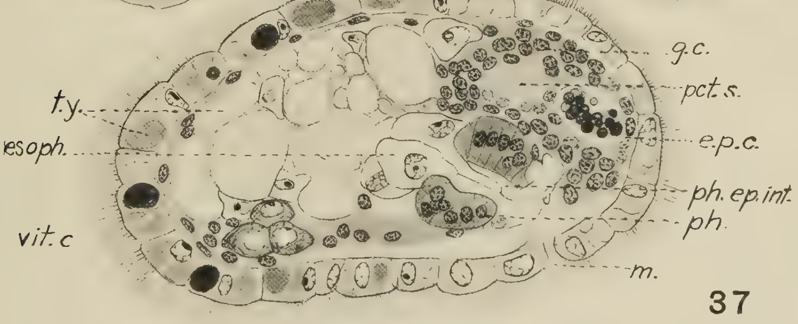

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PLATE 8

EXPLANATION OF FIGURES

42 Frontal section of an embryo somewhat younger than that in figure 37. The yolk masses are seen to be lying inside the secondary entoderm cells, having lost the membranes of the primary entoderm. Mitochondrial mass still visible with its centrosome. Yolk in the upper cell (*t.y.*) has been transformed to a fluid mass. × 730.

43 Sagittal section through an embryo which had not yet left the capsule. Drawn to show the vitellogenous cells migrating posteriorly. × 730.

44 Oblique transverse section through an embryo still in its capsule. Several large vitellogenous cells appear between the entoderm and sub-dermal muscle layer. Above are shown for comparison several cells at the tip of one of the mother's vitellarial strands. × 730.

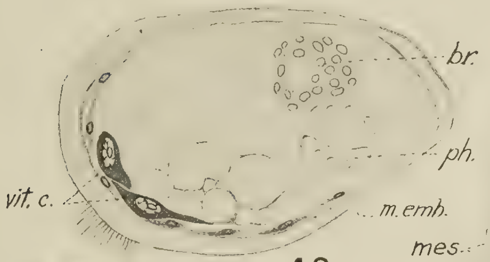
45 Anterior end of an embryo in which the cephalic glands are differentiating. Embryo still in capsule. × 730.

46 Cephalic glands at a later stage showing outlet to the exterior. Embryo free in the mother's mesenchyme. × 730.

47 Oblique frontal section of a young worm 0.30 mm. long found in sea water in which mussels had been washed. Relation of young testis and ovary is shown, as well as the fact that the vitellaria develop from the posterior mass of vitellogenous cells (figs. 43, 44). Cellular nature of the intestine is apparent. Subdermal and intestinal glands, and the structure of the mesenchyme are indicated. × 450.

48 Seminal vesicle of living adult containing several spermatids. × 808.

br., brain
e., eye
ent.1, primary entoderm
ent.2, secondary entoderm
gl.c., cephalic glands
gl.int., intestinal glands
gl.sub., subdermal glands
int., intestine
m.emb., embryonic muscle cell
mes., mesenchyme
mit., mitochondrial mass
o., ovary
ph., pharynx musculature
sp., spermatozoon
spt., spermatid
s.v., vesiculum seminalis
t., testis
vit., vitellarium
vit.c., vitellogenous cells
x., vitellogenous cells in mitosis
y., yolk



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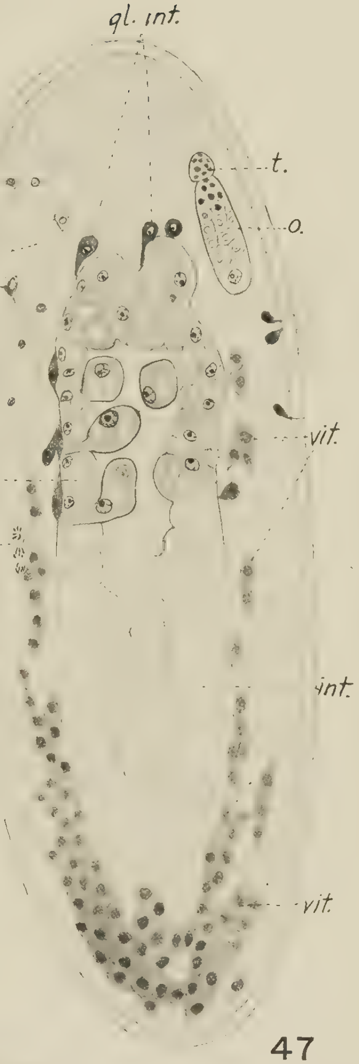

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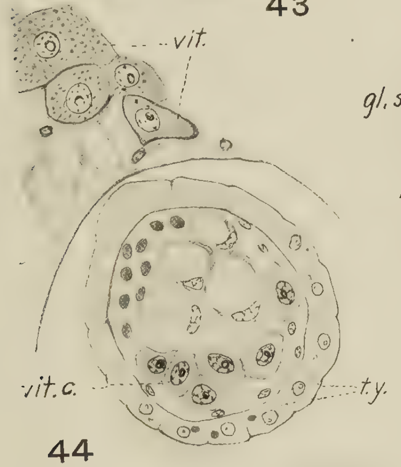

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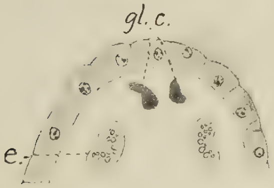

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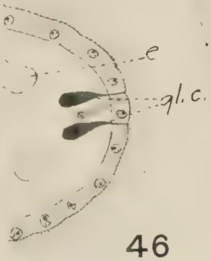

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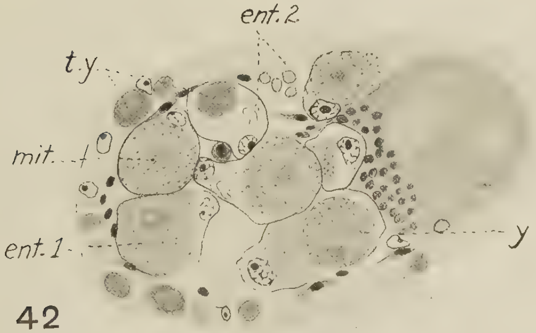

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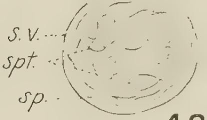

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PLATE 9

EXPLANATION OF FIGURES

49 Lateral view of anterior end of an embryo which had entered the mother's intestine. Drawn to show the gonads with cells in mitosis. × 730.

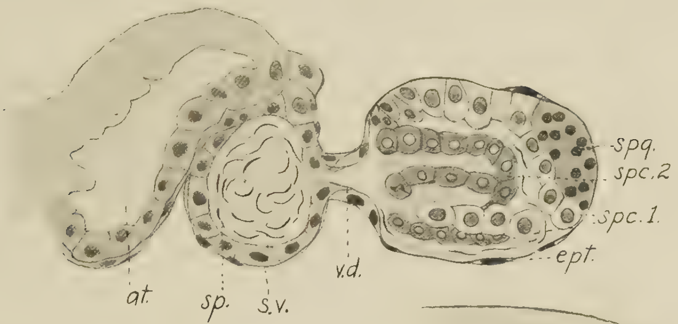
50 Sagittal section through the reproductive organs showing the undifferentiated cell mass which is to give rise to the atrium, antrum femininum and oviduct; also the shell glands (*gl.sh.*). × 400.

51 Sagittal section showing later stage in the development of the sexual organs. Testis has become functional showing spermatogonia, primary and secondary spermatocytes and mature spermatozoa. A number of the latter have already reached the seminal vesicle. Not yet has a lumen been formed in the antral region. × 400.

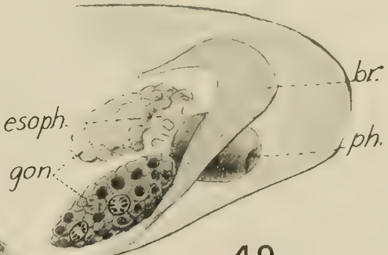
52 Reconstruction from sections of the reproductive organs of a 0.60 mm. worm which had been liberated from the mother for some time. The point of view is one looking posteriorly from a point just in front of the testes. × 270.

53 Oblique frontal section of an abnormal embryo in which the primary entoderm cells, having failed to absorb yolk according to their function, still remain in the center of the unusually small posterior half. The mesenchymatic cells have proliferated as in normal embryos and appear in several rows. × 530.

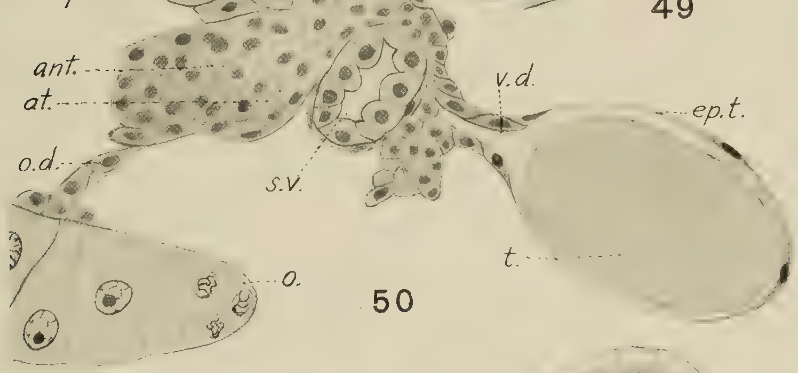
ant., antrum femininum
at., atrium commune
br., brain
ent.1, primary entoderm
ep.t., epithelium of testis
esoph., esophagus
gl.sh., shell glands
gon., gonad
g.p., genital pore
o., ovary
o.d., oviduct
ph., pharynx musculature
sp., spermatozoon
spc.1, primary spermatocyte
spc.2, secondary spermatocyte
spg., spermatogonia
s.v., vesiculum seminalis
t., testis
v.d., vas deferens
vit., vitellarium


spq.
spc.2
spc.1.
ept.
v.d.
at.
sp.
s.v.

51


br.
esoph.
ph.
gon.

49


gl.sh.
ant.
at.
v.d.
ep.t.
o.d.
s.v.
t.
o.

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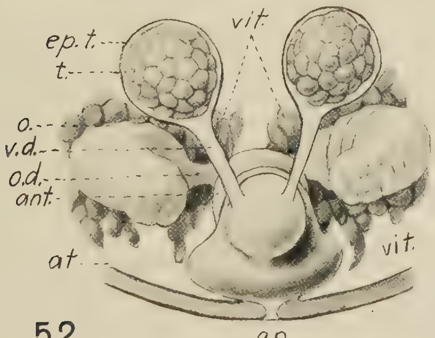
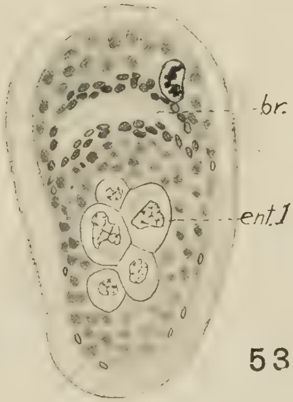

ep.t.
t.
vit.
o.
v.d.
o.d.
ant.
at.
vit.
g.p.

52


br.
ent.1

53

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June, 1916

THE ANATOMY OF A THREE-LEGGED KITTEN

W. B. KIRKHAM AND H. W. HAGGARD

From the Sheffield Scientific School and the Osborn Zoological Laboratory, Yale University

THREE FIGURES

The animal which is the source of this paper was an ordinary, tiger-striped, female kitten which came into the possession of the laboratory through the kindness of our colleague, Dr. Henry Laurens. It lived in the laboratory for a number of weeks, and when about six months old was killed and dissected to determine the exact nature of the anomaly.

The female parent of this kitten had previously given birth to other litters of normal offspring, and this animal which we studied was one of a litter of three, of which the other two were normal. Furthermore, all the members of a litter born since our specimen are normal. The male parent of our kitten is unknown.

The kitten, aside from its having only three legs, was a lively, healthy animal, with sleek fur, and a playful disposition. It balanced itself well, planting the right fore foot under the midline of its body to compensate for the missing left limb (fig. 1). There was no external evidence of any part of the left fore limb except a lump at the shoulder which indicated the presence of at least a part of the shoulder girdle, and the skin was drawn smoothly over the shoulder without any sign of puncture or folding.

As soon as the animal was chloroformed the skin was reflected from the left side of the body, and at once there came to view, beneath a thin layer of fascia, a point of bone (fig. 2). The shoulder muscles (deltoids, clavo-brachial, supra- and infraspinatus, teres major and minor, and subscapular) were all

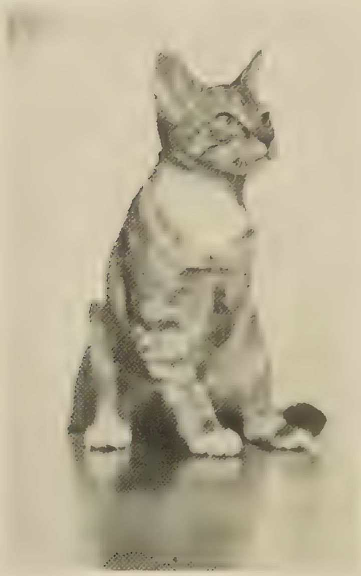

Fig. 1 Photograph of the three-legged kitten when about six months old. The right fore limb is swung under the body to compensate for the missing left limb.

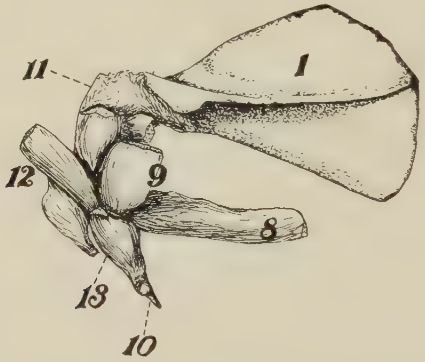

Fig. 2 Left scapula, rudimentary humerus, and deep shoulder muscles of the three-legged kitten. *1*, scapula; *8*, spinodeltoid; *9*, acromiodeltoid; *10*, distal end of the humerus; *11*, capsule of the shoulder joint; *12*, mass of muscle fibers representing the combined latissimus dorsi and the pectorals; *13*, anomalous band of muscle fibers encircling the rudimentary shaft of the humerus. The unnamed stumps of muscles appearing below the joint capsule are those of the supra- and infraspinatus.

normal on the left side of the body with respect to origin, insertion, and innervation. This is not surprising, since the skeletal parts with which they are connected were only slightly abnormal, as described in detail later on.

The appearance of the left fore limb after the removal of most of the shoulder muscles is shown in figure 2. Two muscle anomalies were clearly evident. First, the fibers of the latissimus dorsi, pectoralis major and pectoralis minor muscles, all three of which were thinner than usual, but possessed of normal origins, became fused into a common sheet which was inserted on the inner surface of the humerus, where normally the latissimus dorsi alone is inserted, the normal place of insertion of the pectorals having failed to develop in this humerus. The second muscle anomaly was a mass of fibers which was wrapped completely around the humerus from the point of insertion of the pectorals and deltoids to within a short distance of the tip. Into this mass of muscle ran the musculospiral, musculocutaneous, median and ulnar nerves, but we feel no justification in offering a theory as to the homology of this anomalous mass of fibers. In addition to the above mentioned anomalies should be noted the entire absence of any recognizable triceps, biceps or coracobrachial muscles, a fact to be correlated with the absence of their points of origin from the abnormal left scapula and humerus. Possibly these three muscles are among those represented by the unnamed mass mentioned above.

The right and left scapular and humeral bones of the three-legged kitten are shown, drawn to the same scale, in figure 3. The clavicles, which are not represented, were normal on both sides. The left scapula differs from the right in the following respects: (a) the anterior superior border, owing to the narrow neck, forms a greater angle with the axillary border, (b) the spine of the left scapula, perhaps owing to the undeveloped state of the infraspinatus muscle, is lower than that of the right, (c) the suprascapular notch, on the anterior superior border, is missing, owing to the absence of the coracoid process, (d) the axillary border of the left scapula shows, in correlation with the abnormally narrow neck and head, a decided thickening at its junction with the neck, (e) the neck of the left scapula is only about half the width of that of the right, (f) there is an entire absence from the left scapula of the coracoid process, including the areas for the attachment of the coracobrachialis and biceps muscles and the supraglenoid fossa, (g) correlated with the complete absence of the coracoid process, the glenoid cavity of the left scapula is imperfectly developed, possessing not more than half its usual area, and it is convex rather than concave. In connection with the above mentioned anomalies it should be noted that according to Jayne ('98) the coracoid process and the upper portion of the glenoid cavity of the scapula develop in the cat from distinct ossification centers. Also, the work of Braus ('08) and of Harrison ('15) has shown that in Amphibia the anlagen of the shoulder girdle and of the fore limb are entirely distinct, since experimental removal of the latter does not prevent the development of the former.

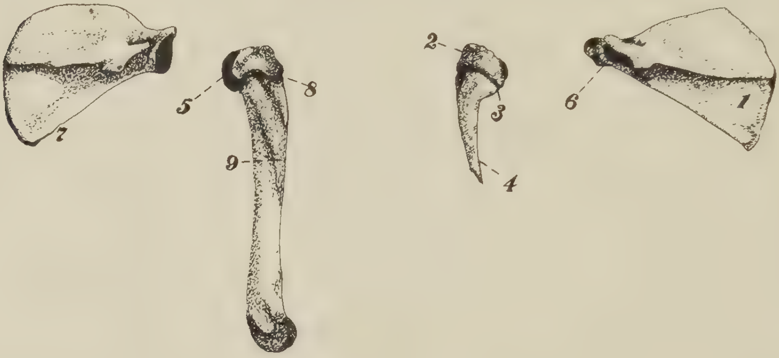

Fig. 3 Outer aspect of the right and left scapular and humeral bones of the three-legged kitten, all drawn to the same scale. *1*, left scapula; *2*, left humerus; *3*, epiphysial groove; *4*, shaft; *5*, right humerus; *6*, abnormal depression in neck of left scapula; *7*, right scapula; *8*, epiphysial groove; *9*, shaft.

The left humerus of this specimen is chiefly characterized by its representing so small a part of the normal bone. Like the right humerus the left shows a clearly defined epiphysial groove, but on the left humerus there is an entire absence of the lesser tuberosity, together with about half of the articular surface adjoining that tuberosity. The part of the shaft present on the left humerus corresponds to the lateral surface of the normal bone from the anterior mid-line laterally to the deltoid ridge. It is everywhere covered with a thick layer of compact bone, and possesses no marrow cavity, its whole interior being filled with spongy bone.

What caused the production of this three-legged animal must probably await conclusive settlement until such anomalies are experimentally controlled, but taking into account all of the available evidence it would appear that after starting to grow out normally from the body the left fore limb bud encountered some obstacle which pressed it against the body, as well as checked its increase in length; such of it as had already grown then underwent further development into bone, muscle, connective tissue, etc.

MARCH 1916

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Überreicht von dem Verfasser

NOCHMALS UEBER DIE MEROGONIE DER OENOTHERABASTARDE

RICHARD GOLDSCHMIDT
Kaiser Wilhelm Institut für Biologie, Berlin

Sonderabdruck aus GENETICS **1**: 348-353, Juli, 1916

NOCHMALS UEBER DIE MEROGONIE DER OENOTHERABASTARDE

RICHARD GOLDSCHMIDT

Kaiser Wilhelm Institut für Biologie, Berlin

[Eingegangen am 20. April 1916]

Im Jahre 1912 veröffentlichte ich eine vorläufige Mitteilung, in der ich zeigte, dass das merkwürdige Erbverhalten der de Vries'schen doppeltreziproken Oenotherabastarde restlos durch die Annahme einer Merogonie dieser Bastarde erklärt wird. Eine vorläufige Untersuchung der Tatsachen schien mir zu beweisen, dass sich die Merogonie auch morphologisch zeigen lässt. Renner, der auf meinen Wunsch hin es unternommen hatte, den Gegenstand weiter zu untersuchen, kam aber zu dem Resultat, dass ich mich getäuscht habe, und bei diesen Bastarden die cytologischen Vorgänge der Befruchtung und Entwicklung normal verlaufen. Da ich nicht glauben konnte, das ich mich derart geirrt habe, beschloss ich, bei erster Gelegenheit den Gegenstand nochmals gründlich zu untersuchen. Das ist nunmehr geschehen und es sei sogleich gesagt, dass ich mich leider überzeugen musste, dass Renner in den meisten Punkten im Recht ist. Es scheint, dass die suggestive Kraft der Merogonieidee meinen Blick bei der ersten Untersuchung trübte, und es bleibt mir nichts übrig als es offen zuzugeben. Die Neuuntersuchung hat mich nun aber mit neuen Bildern bekannt gemacht, die es mir wahrscheinlich machen, dass doch eine Merogonie stattfindet, und so komme ich nochmals auf den Gegenstand zurück.

Von Baur, Renner, Davis u. A. ist an den de Vries'schen Resultaten Kritik geübt worden. Wäre sie berechtigt, dann hätte eine cytologische Untersuchung keinen Sinn. Denn wenn das Erbverhalten nicht den de Vries'schen Angaben entspricht, dann kann es auch nicht mit Merogonie erklärt werden. Mir scheinen allerdings die Einwände wenig stichhaltig zu sein, da sie einmal den noch unbekannten Umfang der plasmatischen Vererbung nicht berücksichtigen (z. B. der Hinweis darauf, dass die F_1 Bastarde nicht rein väterlich sind), oder vergessen, dass die nicht in reinen Linien gezogenen Eltern nicht völlig homozygot sein müssen (der Einwand, dass F_2 nicht völlig konstant sei). Aber trotzdem erschien mir eine Neuuntersuchung nur dann wünschenswert, wenn sie sich auf ein genetisch einwandfreies Material beziehen konnte. Dies wurde mir nun durch die grosse Liebenswürdigkeit von G. H. SHULL ermöglicht, der sich der Mühe unterzog, in seinem Pedigreematerial die nötigen Kreuzungen auszuführen und mir ein sehr umfangreiches Material zu beschaffen. Es ist mir eine angenehme Pflicht ihm dafür herzlichst zu danken, und ebenso auch Dr. METZ, der die Conservierung nach meinen Angaben ausführte.

Dr. SHULL ermächtigt mich die folgenden brieflichen Angaben über die betreffenden Bastarde zu zitieren:

> "The two species of which I sent you pure-bred material and reciprocal crosses were *Oenothera atrovirens* SHULL and BARTLETT and *Oe. venosa* SHULL and BARTLETT. You will find these two species fully described taxonomically, with illustrations, in the AMERICAN JOURNAL OF BOTANY, **1**: 239-242, May, 1914. Unfortunately I cannot refer you to any published account of the hybridization experiments, involving these two species, but the facts are briefly as follows: The hybridization phenomena in these two species represent a typical case of the kind described by DE VRIES as 'double reciprocal hybrids.' Specifically the F_1 progenies are uniform, but quite distinct from one another. With respect to the type of branching and form of buds the F_1 hybrids are strongly patroclinous, but in pigmentation they are just as strongly matroclinous. The F_1 hybrids breed true to their hybrid characters. If we let *Oe. atrovirens* be represented by a, *Oe. venosa* by v, the F_1 hybrids produced by a × v by N, and those produced by v × a by O, the total results of my extensive genetical experiments with these two species may be described as follows:
>
> a × self = a
> v × self = v
> N × self = N
> O × self = O
> a × N = N
> a × O = a
> v × N = v
> v × O = O
> N × O = a
> O × N = v
>
> "No new forms appeared in the second generation of this cross, and the identity of the several types as indicated by the above symbols was clear enough to be beyond question.
>
> "I should add that your material was all guarded, castrated and crossed by my own hands with the precautions I observe in all my genetical work."

Es kann somit keinem Zweifel unterliegen, dass mein Material sich auf genetisch einwandfreie doppeltreziproke Bastarde bezieht. Ich habe nun die ersten Vorgänge im befruchteten Ei nochmals verfolgt und mich davon überzeugen müssen, dass sie völlig normal verlaufen. Es ist mir jetzt auch gelungen, eine Aequatorialplatte der 1. Teilung nach der Befruchtung von der Fläche gesehen zu finden und es kann an der diploiden Chromosomenzahl kein Zweifel sein. Bei der weiteren Untersuchung junger Stadien fand ich aber in der ersten Teilung des Embryos Besonderheiten, die es mir sehr wahrscheinlich machen, dass hier eine Chromatinelimination stattfindet, die denn im Endeffekt das gleiche wäre wie eine Merogonie. Trotzdem sämtliche Stadien der kritischen Epoche, die sich in den Präparaten fanden, Andeutungen des Vorganges aufwiesen, muss ich doch zugeben, dass sie nicht als definitiv beweiskräftig betrachtet werden können. Bei der Wichtigkeit des Gegenstandes wäre es wünschenswert, dass mindestens 100 mal das entscheidende Stadium, die späte Telophase der 1. Teilung beobachtet würde. Vielleicht lässt sich das einmal später ausführen, bis dahin kann man nur sagen, dass ein starker Verdacht besteht, dass die Merogoniehypothese doch das Richtige trifft, obwohl sie durch meinen früheren zu raschen Optimismus jetzt diskreditiert erscheint.

Die Tatsachen sind die Folgenden: nach der Befruchtung verläuft alles völlig normal bis zur Telophase der 1. Teilung. In der späten Telophase zeigt sich nun eine deutliche Verschiedenheit zwischen den beiden Polen. An dem dem Suspensor zuliegenden Pol, der der späteren Fusszelle entspricht, gehen alle Vorgänge normal weiter; an dem Embryopol dagegen ordnen sich die Chromosomen in zwei distinkte Gruppen. Fig. 1 (Tafel 4) zeigt das jüngste derartige Stadium.[1] Eine Gruppe zusammengeklumpter Chromosomen, von einem hellen Hof umgeben, liegt hoch im Präparat, darunter eine zweite Gruppe noch isolierter Chromosomen. Ich möchte dies als Gonomerie deuten. Fig. 2 zeigt ein etwas späteres Stadium. Es ist schräg vom Eipol hergesehen, und die beiden in die Figur eingezeichneten Chromatingruppen liegen somit übereinander in verschiedenen Ebenen. Unten ist der Fusszellenpol sichtbar und hier haben sich die Chromosomen bereits in eine Gruppe typischer Karyomeriten umgewandelt. Am Eipol aber sieht man die in der Entwicklung noch nicht so weit vorgeschrittenen Chromosomen in zwei distinkten Gruppen. Eine genaue Zählung ist aber nicht mehr möglich. In Fig. 3 haben wir nun eine späte Telophase. Die Differenz zwischen dem Eizellpol und dem Fusszellpol ist wieder deutlich. An letzterem ist der Kern schon fast aufgebaut, ersterer zeigt noch die Gruppe von Karyo-

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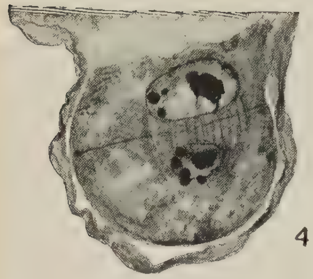
[1] Sämmtliche Figuren wurden von Frl. LINA KRAUSE gezeichnet.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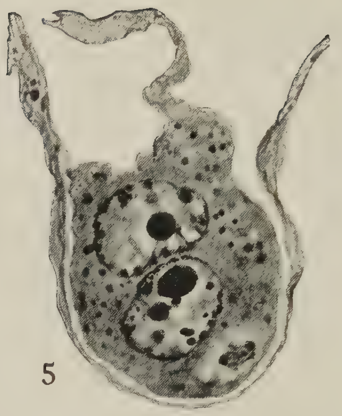
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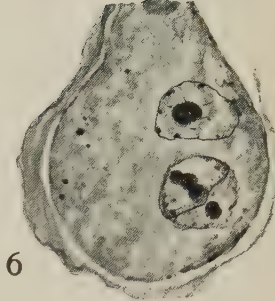
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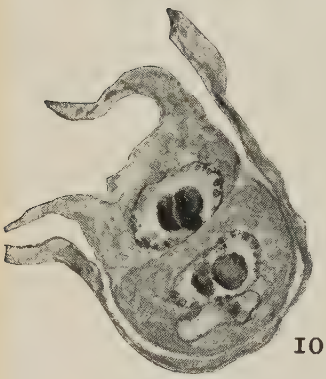
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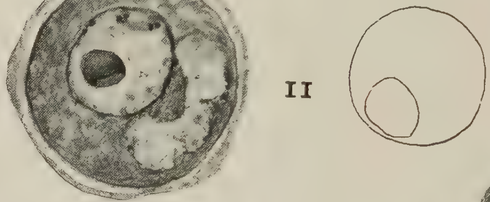
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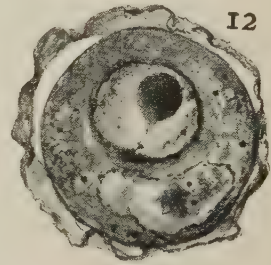
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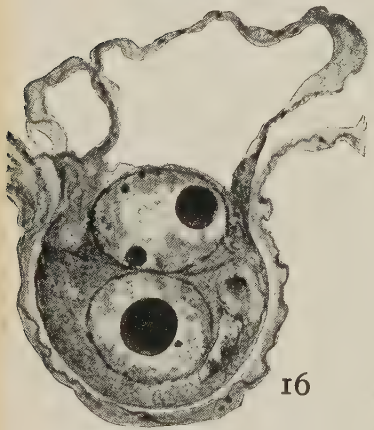
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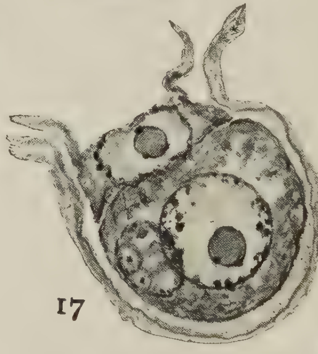
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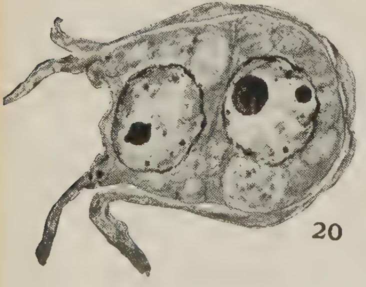
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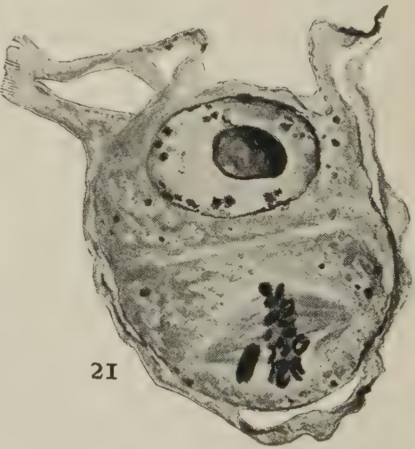
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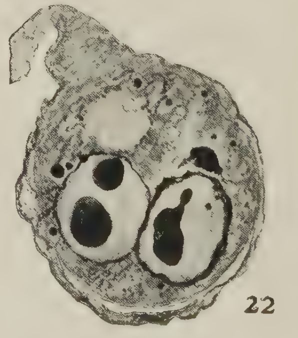
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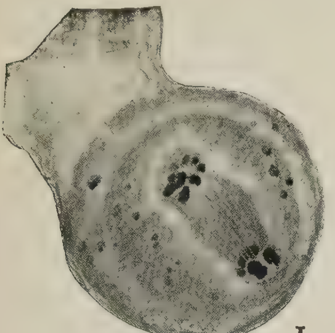
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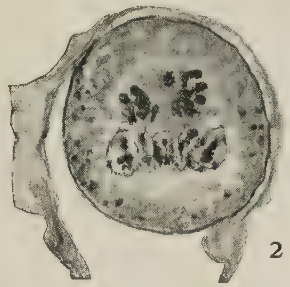
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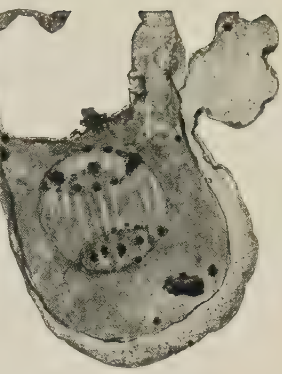
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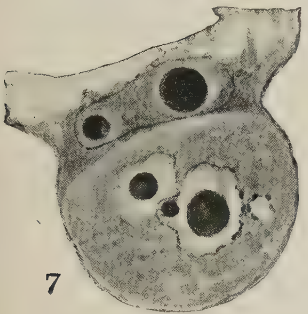
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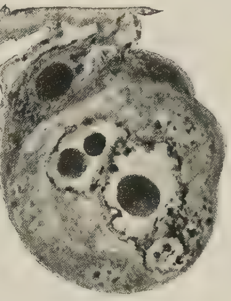
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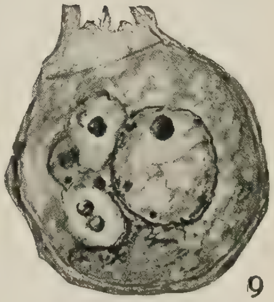
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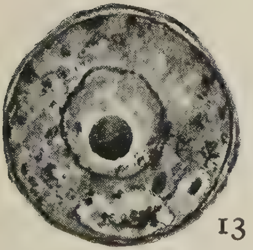
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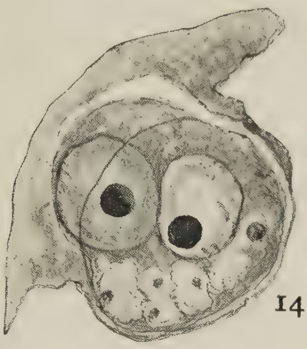
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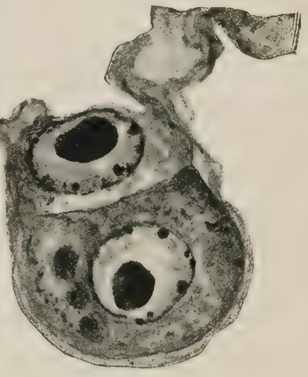
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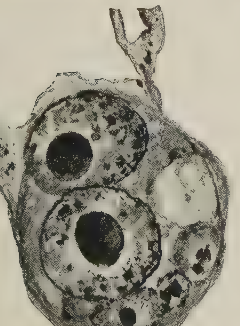
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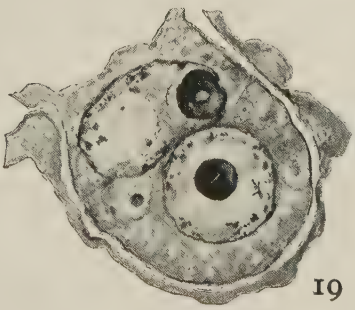
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meriten intakt. Daneben aber liegt im Plasma in einem hellen Hof eine zusammengeklumpte Chromatinmasse. Noch etwas älter ist Fig. 4 Die Scheidewand ist gebildet und der Fusszellenkern fast fertig. Der Embryokern ist aber noch klein und undeutlich und über ihm im Plasma liegt eine Gruppe chromatischer Kügelchen. Ein etwas späteres Stadium in dem aber die Scheidewand nicht deutlich ist, giebt Fig. 5 wieder. Neben dem Embryokern liegt wieder die chromatische Masse in einem hellen Raum.

Es erscheint mir nun nicht unwahrscheinlich, dass diese Bilder folgendermassen zu deuten sind: In der Telophase tritt eine Trennung der väterlichen und mütterlichen Kernsubstanzen ein, wie dies bekanntlich ja auch bei gewissen von HERBST untersuchten Seeigelbastarden stattfindet. Und dann wird bei der Kernrekonstruktion das mütterliche Chromatin dem Untergang geweiht, wofür auch bis zu einem gewissen Grad Beispiele bei den Seeigelbastarden vorliegen. Der Vorgang findet nur in der Embryozelle, nicht in der Fusszelle statt, was uns im Hinblick auf bekannte Vorgänge des Zellenlebens (Diminution von Ascaris) nicht so absonderlich erscheint. Die Merogonie dieser Bastarde wäre also eigentlich nichts anders als die wohlbekannte (BALTZER, HERBST) Chromatinelimination patrokliner und matrokliner Seeigelbastarde, eine Lösung die ich, wie in meiner ersten Mitteilung schon gesagt, von Anfang an erwartet hatte, von der ich aber durch die falsch interpretierten Stadien mit dem degenerierenden Kern abgelenkt wurde.

Für die Wahrscheinlichkeit dass wir uns hier auf der richtigen Spur befinden, sprechen nun zahlreiche Bilder, die sich in den jungen Zweizellstadien finden. So findet man häufig in der Embryozelle den frischrekonstruierten Kern doppelt, woran sich dann Bilder anreihen, in denen neben dem Kern Gebilde liegen, die sich als die Reste der in Auflösung begriffenen Kernhälfte deuten lassen. Fig. 6 zeigt ein junges Stadium in dem die Scheidewand noch undeutlich ist. Der Kern besteht aus zwei übereinanderliegenden Bläschen. In Fig. 7 und 8 haben wir zwei schräg geschnittene Embryonen in denen die Fusszelle nur angeschnitten ist. In der Embryozelle liegen zwei unregelmässig geformte Kernhälften. Daran schliessen sich nun die zahlreichen Bilder, in denen in jüngeren Zweizellstadien neben dem Kern Bildungen liegen, die man leicht als die zu Grunde gehenden Reste der einen Kernhälfte ansprechen könnte. Fig. 9—20 stellen solche Bilder dar, die sich im Wesentlichen selbst erklären. Wo die Fusszelle nicht sichtbar ist, liegt entweder ein Schrägschnitt oder Querschnitt vor, oder der Embryo ist in einem dicken

Schnitt vom Pol gesehen und nur die Ebene der Embryozelle gezeichnet. Es sei dazu eigens bemerkt, dass von irgendwelchen Degenerationen nicht die Rede sein kann, da ich in den späteren Stadien—im Gegensatz zu dem früheren Material—nicht ein einziges Stadium abnormer Entwicklung fand. Die in Frage kommenden Bildungen bestehen in mehr oder weniger scharf begrenzten, mehr oder weniger kernartigen Vakuolen in denen sich chromatische Reste verschiedener Art finden, die um so undeutlicher werden, je älter das Stadium. In diesen Bildern wird auch sehr oft eine beträchtliche Grössenverschiedenheit zwischen dem Embryo- und Fusszellenkern festgestellt, indem ersterer wesentlich kleiner ist. Es ist dies deutlich in Fig. 11, wo die Umrisse des Fusszellenkernes neben dem vom Pol gesehenen Embryo gezeichnet sind, wobei auch die Grössendifferenz der beiden auffällt.[2] Dasselbe zeigt Fig. 14 und besonders 19 und wurde in etwa der Hälfte der zahlreichen untersuchten zweizelligen Embryonen festgestellt. Es ist besonders beachtenswert, weil der Embryokern sich vor dem Fusszellkern wieder teilt und wenn sein Teilungswachstum eingetreten ist, viel grösser als der letztere wird.

Diese Tatsachen und ihre mutmassliche Deutung erhalten nun noch eine weitere Stütze durch folgenden Befund. In der zweiten Zellteilung, die die Embryonenzelle in zwei Halbkugeln zerlegt, findet sich wieder die diploide Chromosomenzahl. In einem in Fig. 21 wiedergegebenen Fall, lag nun ausserhalb der Spindel ein grosses Chromosom ganz isoliert. Es hatte aber die Grösse der Chromosomen der 1. Teilung oder war sogar grösser. Der Gedanke ist naheliegend, besonders im Hinblick auf ähnliche Fälle bei den Seeigelbastarden,—das hier ein mütterliches Chromosom nachträglich eliminiert wird, das bei der ersten Elimination nicht entfernt worden war. Tatsächlich fand sich denn noch ein Embryo kurz nach dieser Teilung mit einem eliminierten Klumpen Chromatin neben dem einen Kerne. Er ist (im Querschnitt der die Fusszelle nicht zeigt) in Fig. 22 abgebildet.

Die berichteten Tatsachen sind nun zweifellos, obwohl an einem recht umfangreichen Material gewonnen, noch nicht genügend, um die Elimination des mütterlichen Chromatins und somit die Merogonie mit Sicherheit zu erweisen. Die Entscheidung muss somit weiterer Untersuchung überlassen bleiben. Bis dahin kann aber die Merogonie der doppeltreziproken Bastarde, die ja die genetischen Erscheinungen restlos erklären würde, keinesfalls als abgetan betrachten werden.

---

[2] Es sei hier bemerkt, dass der Nucleolus ein Chromatinnucleolus ist und in der Prophase an dem Aufbau der Chromosomen teil nimmt. Ich habe selten schönere Bilder dieses Vorgangs gesehen als bei diesen Embryonen.

Osborn Zoological Laboratory,
New Haven, Conn.
Im März, 1916

ZITIERTE LITERATUR

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September, 1916

THE PROLONGED GESTATION PERIOD IN SUCKLING MICE

WILLIAM B. KIRKHAM

From the Sheffield Scientific School and the Osborn Zoological Laboratory, Yale University

INTRODUCTION

A short paper by Prof. J. F. Daniel ('10) on white mice, and a later paper by Dr. H. D. King ('13) on white rats, have shown that in these two animals the gestation period is lengthened when the females suckle young during pregnancy. Daniel found a fairly definite relation existing in mice between the degree of prolongation of the gestation period and the number of young being suckled, about one day being added to the period of pregnancy for each of the nursing young. King, on the contrary, found that in rats the gestation period is normal "if the female is suckling five or less young and is carrying five or less young," while "the gestation period may be prolonged from one to six days if an albino female, suckling five or less young, is carrying six or more young," and "the period of gestation is always prolonged when a female is suckling six or more young."

Professor Daniel offered no explanation of the prolongation of the gestation period in suckling white mice other than stating the possibility that it might be due to lactation, either delaying ovulation or generally slowing down embryonic development, with the latter as the more probable. Dr. King, however, states positively that ovulation in albino rats is not delayed by lactation, but that the suckling of a litter that contains six or more young seems to lessen the food supply to the foetal young and so retards their development.

The present work has been in progress for nearly five years, and certain phases of it are still being investigated. White mice only have been used, and the problem of prolonged gestation in suckling females has been approached from the embryological standpoint in the attempt to determine the detailed facts involved.

It was evident at the start that exact criteria for determining the stage of development of any embryo must be established, and, while Sobotta ('95) gives the time relations of development from ovulation to implantation, the series of eggs and embryos of known age prepared especially for this work includes sets from both suckling and non-suckling females, spaced at about twenty-four hour intervals from the time of parturition to the birth of the following litter. In each case the age of the specimens was definitely established by placing in individual cages all pregnant females, together with a male, looking every day for new litters, removing the male in every instance the day following the birth of the litter, and, if the female was to be used for the non-suckling series, removing all the young as soon as found. The age of eggs and embryos found in these females was then reckoned from the birth of the preceding litter, e.g., the day the litter was found is the first day after parturition.

THE DEVELOPMENTAL CYCLE IN NON-SUCKLING FEMALES

The development, in non-suckling white mice, of eggs and embryos is usually as indicated below, the exception being the very rare instances where the males probably paired with the females much earlier than the average time, following parturition, and in consequence the developing eggs or embryos are slightly ahead of this schedule. Cases of this kind number only three in eighty sets of eggs and embryos studied. It should be noted, further, that as a rule (one exception in fifty instances) when the males were left with the females the state of development of eggs and embryos was the same as in cases where the males were removed twenty-four hours after parturition. The single exception found was a set of normal eggs in the two-cell stage found in a female six days after parturition, a male having been in the cage with her continuously since a time preceding the birth of the litter.

Parturition. White mice give birth to litters at all hours of the day and night, but much more commonly at night or, according to Long and Mark ('11), in the early morning.

1 day post-partum. If the female white mouse comes in heat, as is the rule during the warmer part of the year (April to October) and the new born young are not suckled, within twenty-four hours after parturition ovulation and pairing (provided a male is present) will occur.

2 days post-partum. The eggs have been fertilized in the upper third of the Fallopian tubes and the first cleavage has occurred.

3 days post-partum. The eggs are still in the two-cell stage.

4 days post-partum. Cleavage is again in progress and morulas of 8 to 10 blastomeres are found at this time.

5 days post-partum. Morulas of 12 to 16 blastomeres. At the close of this day the morulas develop a central cavity, thus becoming blastulas, and at the same time they pass from the Fallopian tubes into the horns of the uterus.

6 days post-partum. Blastodermic vesicles lie free in the horns of the uterus.

7 days post-partum. The blastodermic vesicles are now implanted in proliferated masses of uterine cells which completely obstruct the lumen. The embryos themselves are in the 'egg-cylinder' stage, the 'cylinder' almost filling the vesicle and possessing a single, undivided cavity.

8 days post-partum. The egg-cylinder in embryos of this age has its lumen divided into three cavities.

9 days post-partum. The embryo now possesses a medullary groove, which is open except at the extreme anterior end.

10 days post-partum. Embryos of this age have the medullary groove closed in its anterior half, and for the first time show a heart.

11 days post-partum. The medullary groove is now closed for more than half its length; the optic vesicles are budding off from the brain; and the auditory vesicles appear as cup-shaped depressions in the ectoderm.

12 days post-partum. The medullary groove has closed except at the extreme posterior end; the auditory vesicles are almost or entirely closed. The fore limb buds are present, together with the first nephric tubules and the anlage of the liver.

13 days post-partum. At this age the embryo is a decidedly complex organism, and from this time on the daily changes are rather matters of detail than the appearance of entirely new structures. The characteristic features of this particular stage of development are these: a well developed cranial flexure; optic vesicles completely separated from the brain, invaginated, and showing the beginnings of lens formation. The nasal capsules are visible; the liver has developed into a distinct organ; hind limb buds are present. The embryo has the anlagen of the lungs, and a few pancreatic tubules; the auditory vesicles have withdrawn from the surface and are connected by nerve fibers with the brain. Along the free border of the kidneys, especially at the posterior end appears the genital ridge with a few large cells with large round nuclei, the primordial germ cells, scattered through a much larger number of smaller, epithelial cells.

14 days post-partum. The eyes have developed to the stage where the lenses have a solid, clear core. Dense masses of connective tissue foreshadow the future location of the bones of the limbs, girdles, and ribs. Whisker follicles are present; also the semi-circular canals of the ears. The kidneys possess definite boundaries. The nuclei of the red blood corpuscles are smaller and stain less deeply than in earlier stages, while their cytoplasm shows a faint indication of haemoglobin. The first indications of teeth follicles are found in embryos of this age; also the anlagen of the thymus and thyroid glands. The gonads differ from the preceding stage merely in having more of the primordial germ cells in the genital ridges.

15 days post-partum. Embryonic cartilage cells constitute the most striking characteristic of this stage, clearly differentiating embryos of this age from all younger specimens. Other features are the fewer and smaller blood spaces in the liver, as compared with fourteen-day embryos; the deeper straw yellow color in the cytoplasm of the red blood corpuscles, together with a few which are non-nucleated; the well-developed anlagen of the teeth; and the presence of cartilage cells in the floor of the cranium. Sexual differentiation is present in embryos fifteen days post-partum, male specimens showing gonads in which follicle formation has already started, while the female gonads preserve the earlier condition of primordial germ cells scattered through a mass of epithelial cells.

16 days post-partum. Differential characteristics now become still more matters of detail and of direct comparison with earlier stages, however, embryos of this age differ from all younger ones in having a decidedly transparent cornea. The heart has assumed its final shape. The pancreas is a clearly defined organ. The nasal capsules open into the front part of the mouth, while the naso-pharynx is connected with both the nasal capsules and the back part of the mouth. The gonads show no marked change from those of fifteen days embryos.

17 days post-partum. The chief characteristic of embryos of this age is the commencement of ossification around the rib cartilages. Nucleated red blood corpuscles are very scarce. The tongue possesses conspicuous striated muscle cells, stratified epithelium, and at least one circumvallate papilla. The nasal capsules have lost their direct connection with the mouth, but the nasopharynx opens into both the anterior and posterior regions of the mouth. The anlagen of the cartilaginous rings of the trachea are present. The testes have tubules with a peripheral layer of small cells while the larger primordial germ cells occupy the lumen. In the ovaries an ingrowth of connective tissue is noticeable.

18 days post-partum. Ossification of the cartilaginous skeleton is now the striking feature, and the membrane bones of the upper jaw, hard palate and roof of the skull are also being formed. The testes show a considerable amount of connective tissue between the tubules, while the ovaries differ from those of the preceding stage only in that they project further into the abdominal cavity.

19 days post-partum. The ribs of embryos of this age have an outer shell of bone, and the underlying cartilage is being torn down to make a marrow cavity. There is a marked spongy structure in the lungs; the eye balls show a differential curvature in the cornea and sclerotic; the naso-pharynx no longer opens into the front of the mouth. The testes show no change, but the ovaries are more spherical, and the ingrowth of connective tissue has forced most of the germ cells toward the periphery.

20 days post-partum. The iris and choroid of the eyes first show pigmentation at this time, and lymphoid tissue appears in the tonsils. One litter of four animals was born twenty days after the birth of a preceding litter and grew to maturity.

21 days post-partum. The feature of this stage of development is the ossification of the metacarpals and metatarsals. The testes have a well organized tunica albuginea and show less space between the individual tubules than in twenty-day specimens, while in the ovaries the primordial germ cells, or oogonia, are of varying sizes, the largest of them beginning to form follicles about themselves.

22 days post-partum. The embryos in non-suckling white mice have now completed their intra-uterine development, and parturition occurs.

Such, in brief, is the record of development from day to day of white mouse embryos carried by females not suckling young. More extended study and more material would certainly yield many more details, but the above data are sufficient basis for estimating the age of embryos of unknown history, provided only they come from non-suckling females. Also the evidence collected is adequate for comparison with the facts noted below concerning the development of embryos carried by suckling mothers.

THE DEVELOPMENTAL CYCLE IN SUCKLING FEMALES

White mice are able to become pregnant while lactating but when suckling a litter only about one female in five undergoes a complete pregnancy; those which do not complete a pregnancy either failing to ovulate (the majority of cases) or the fertilized eggs developing normally until shortly after implantation in the uterus, when they die and are absorbed (the minority of cases). In some instances we find another state of affairs, certain ones of a set of implanted embryos undergoing normal development while others die and are absorbed, a very rare condition in non-suckling females.

Suckling females which are not going to skip an ovulation cycle shed their eggs within twenty-four hours of parturition as do non-suckling females. These eggs are then fertilized and divide according to the same time scheme as given above for eggs in non-suckling females, being in the two-cell stage on the second and third days following parturition, morulas on the fourth and fifth days, and blastulas lying free in the uterus on the sixth day post-partum.

Now comes the point of greatest interest in this investigation. The fertilized eggs in non-suckling females, as stated earlier in this paper, become implanted in the uterus at the close of the sixth day post-partum, the fertilized eggs in suckling white mice, on the contrary, lie free in the lumen of the uterus from the sixth to the end of the fourteenth day following parturition. The material on which this statement is based comprises serial sections of the entire uterus of ten females, suckling from three to eight young, killed at various times from the sixth to the fourteenth day post-partum, all of which show normal blastulas lying free in the uterus, with no sign of any reaction in the adjacent cells of the uterine epithelium (table 1).

TABLE 1

Data regarding all suckling white mice from which embryos were obtained from the sixth to the fourteenth day following parturition

AGE IN DAYS POST-PARTUM	STAGE OF DEVELOPMENT	NUMBER OF SUCKLING YOUNG
6	Free blastulas in uterus	6
7	Free blastulas in uterus	4
8	Free blastulas in uterus	5
9	Free blastulas in uterus	3
10	Free blastulas in uterus	5
10	Free blastulas in uterus	8
11	Free blastulas in uterus	6
12	Free blastulas in uterus	4
13	Free blastulas in uterus	4
14	Free blastulas in uterus	5

Here evidently is one, and perhaps the main cause of the prolonged gestation in suckling white mice. Ovulation, fertilization and early cleavage stages occur at the same time intervals in both suckling and non-suckling animals, but in the suckling females the uterine cells will not react to the eggs and enable the latter to become implanted while the mammary glands are withdrawing the surplus nourishment from the parent organism. In support of this statement it should be said that young mice derive all of their nourishment from the mother for the first ten or eleven days after they are born, and thereafter appear to nurse as much and as often as the female will let them, a fact which undoubtedly accounts for the precise number of suckling young exerting a definite influence on the rate of growth of intrauterine embryos.

If only one or two young mice are suckling the development of eggs and embryos proceeds as though none were suckling, but if three or more young nurse the gestation period is lengthened, according to both Daniel ('10) and to the small amount of evidence on this point possessed by the present writer, approximately one day for each animal suckled. This observed fact is, however, very difficult to correlate with the series of embryos obtained from females suckling three to eight young and killed from fifteen to twenty-four days after parturition (table 2). These embryos show a variation in state of development which appears to vary neither with the number of young suckled nor with the number of embryos carried; in fact it would seem as though in one instance (female killed eighteen days post partum table 2) that the embryos, being as fully developed as in a non-suckling female of the same age post-partum, would have come to birth on the twenty-second day following the previous parturition in spite of there being three young suckling. Even supposing this had happened, were all the facts known such an exception would probably be explainable, since occasional females may reasonably be expected to possess amounts of nourishment far in excess of the average, and some litters of young may start eating grain at an earlier age than usual. More difficult of explanation are such cases as the embryos of a female killed seventeen days

TABLE 2

Data regarding all suckling white mice from which embryos were obtained from the fifteenth to the twenty-fourth day following parturition. Embryos labeled (small) would probably all have been absorbed

AGE IN DAYS POST-PARTUM	DEVELOPMENT EQUAL TO EMBRYOS OF NON-SUCKLING ♀S OF	NUMBER OF SUCKLING YOUNG	NUMBER OF EMBRYOS
	days p. p.		
15	8	8	11
16	12	4	8
17	14	5	2 large + 6 small
18	18	3	9
19	12	7	2 large + 4 small
20	8	6	5
21	8	5	9
21	12	8	9
22	8	?	10
23	12	7	9
24	14	4	6 large + 1 small

post-partum (table 2) which, if, as we have every reason to believe, they became implanted at the close of the fourteenth day post-partum must in the course of the three days following have undergone a development which in embryos in non-suckling females requires eight days to complete, and this in spite of there being five suckling young.

At the present time work is in progress with a view to explaining, if possible, these apparent contradictions and until that work is completed, which may not be for some time, it does not seem desirable to attempt any further analysis of the facts presented in table 2.

SUMMARY

1. The present work has brought together sufficient data with which to determine, within the possible error of one day, the age of all embryos obtained from non-suckling white mice.

2. Ovulation, fertilization, and the early cleavage of the eggs bear the same time relations to parturition and to one another in both suckling and non-suckling white mice except the former are much more apt to skip an ovulation period.

3. Implantation of embryos in the uterus occurs in non-suckling white mice on the fifth day following parturition (provided the female did not skip an ovulation cycle).

4. Implantation of embryos in the uterus occurs in suckling white mice, with 3 or more young, on the fourteenth day following parturition (provided the female did not skip an ovulation cycle). In these lactating females the blastulas lie free in the lumen of the uterus from the sixth to the fourteenth day post-partum due supposedly to the activity of the mammary glands.

5. The available material of stages following implantation in suckling females shows no evident correlation with either the number of nursing young or the number of embryos being carried. It also is impossible at present to reconcile the development of these embryos with the observed facts regarding the time of parturition in suckling mice.

6. The conflicting evidence from post-implantation stages in suckling females is at present being subjected to further study.

JUNE, 1916

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THE FUNCTION OF THE APYRENE SPERMATOZOA

SINCE the discovery of the dimorphism of spermatozoa in *Paludina* by von Siebold, 1836, numerous biologists have worked with this strange phenomenon without being able to find a conclusive explanation. On the one side investigators were found, like v. Brunn, who regarded the abnormal type of spermia as abortive elements without any function, whereas others, like Brock and Auerbach, thought it impossible that such typical elements could be produced regularly without being functional. It is well known how a new basis was given to these discussions, when Meves (1903)[1] worked out the details of the spermatogenesis of these two types of spermatozoa. He first proved that the atypical spermia in Prosobranchs contain only a small part of the chromatin of the cell and called them *oligopyrene* spermatozoa. He further discovered a similar dimorphism in some moths, but here the atypical spermia contain no chromatin at all, they are apyrene. Meves could get no evidence regarding the possible function of these elements, but he felt sure that they must have some function and pointed to the possibility of a fertilization by these elements, which would be interpreted as an "Entwicklungserregung." Since then much morphological work upon the structure and development of the atypical elements has been done, especially by Retzius, Kuschakewitsch, Reinke,[2] which, however, does not interest us here.

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[1] Meves, F., "Ueber oligopyrene und apyrene Spermien und ueber ihre Entstehung nach Beobachtungen an Paludina und Pygaera," *Arch. Mikr. Anat.*, 61, 1903. See there the quotations of the previous literature.

The interest in the possible function of these elements was awakened again, when the facts about the sex-chromosomes became known and their relation to the other kind of dimorphism of spermatozoa in insects and the mechanism of sex inheritance. The idea was promoted by R. Hertwig[3] that this dimorphism might also be connected with sex-determination and he tried to fit these possibilities into his general ideas of sex-determination, considering the possibility of fertilization by apyrene spermatozoa as a kind of male-producing parthenogenesis. These ideas were the starting point of some work which Popoff[4] did with *Paludina.* But he was unable to prove that the oligopyrene spermatozoa of that snail take any part in fertilization, although they are found in sufficient numbers in the oviduct. The only positive result was that in impregnated snails the oligopyrene spermia degenerate and die much earlier than the normal ones. Lamas,[5] who later studied the fertilization of *Murex,* was also unable to find any such spermatozoon inside the egg. On this point we have only a single positive observation given by Kuschakewitsch.[6] He found in the eggs of a prosobranch, *Aporrhais,* twenty minutes after fertilization, an oligopyrene spermium besides a typical one. Even taking it for granted that this is not an accident of sectioning, the facts are not yet convincing. And Kuschakewitsch himself is indeed rather skeptical and does not want to draw far-reaching conclusions. It might be mentioned that we had formulated a hypothesis[7] of sex-determination on the basis of such a process which, however, we have since abandoned. The latest work on these questions, by Reinke (*l. c.*) finally reached one positive, but rather discouraging result. He finds in *Strombus* that the atypical spermatozoa never reach the receptaculum seminis but degenerate and are surrounded by a capsule. The simultaneous work of von Kemnitz[8] also failed to attain positive results, although the fact is of interest that the hermaphroditic prosobranch *Valvata* exhibits no dimorphism of spermatozoa.

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[2] Retzius, G., *Biol. Unters.*, N. F., Vol. 12, 13, 14, 1905–1909. Kuschakewitsch, S., ''Studien über den Polymorphisms der männlichen Geschlechtselemente bei den Prosobranchia,'' *Arch. Zellforschg.*, 10, 1913 (complete literature). Reinke, E. E., ''The Development of the Apyrene Spermatozoa of *Strombus tuberculatus,*'' Publ. 183, Carnegie Inst., Washington, 1914.

[3] Hertwig, R., ''Ueber Correlation von Zell- und Kerngroesse, etc.,'' *Biol. Centrbl.*, 23, 1903.

[4] Popoff, M., ''Eibildung bei Paludina vivipara, etc.,'' *Arch. mikr. Anat.*, 70, 1902.

[5] Lams, H., ''Recherches concernant le dimorphisme des élements sexuels chez le Murex,'' *Ann. Soc. Med. Gand.*, 89, 1910.

As far as we know, only once has a real experiment been performed to test the function of the apyrene spermatozoa. R. Hertwig[9] started from the hypothesis that fertilization with apyrene spermia is comparable to the parthenogenesis and produces males. Therefore he crossed two species of moth, *Pygæra anachoreta* and *curtula,* which are known to produce apyrene spermia. If fertilization could occur by these latter sex-cells, the offspring, supposedly the males, ought to exhibit only maternal characters. The results were entirely negative, both sexes in F_1 being intermediate in regard to the characters of the parental species.

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[6] Kuschakewitsch, S., "Zur Kenntnis der sogenannten wurmförmigen Spermien der Prosobranchier," *Anat. Anz.*, 37, 1910.

[7] Goldschmidt, R., "Kleine Beobachtungen und Ideen zur Zellenlehre," I., *Arch. Zellforschg.*, 1910.

[8] V. Kemnitz, G., "Beiträge zur Kenntnis des Spermatozoendimorphismus," *Arch. Zellf.*, 12, 1914.

[9] Hertwig, R., "Ueber den derzeitigen Stand des Sexualitaetsproblems, etc.," *Biol. Centrbl.*, 32, 1912.

We are now able to state a few experimental facts in regard to the apyrene spermia of moths which have been noticed in connection with some other work. The first question is are the apyrene spermia able to fertilize an ovum or to cause development? Some answer is given by the following facts. In my experiments on intersexuality in the gipsy-moth an almost complete series of intersexual males was produced in 1915 showing every stage from a male to a female. Now up to a certain degree of intersexuality these individuals behave sexually like males and succeed in mating with the females. For many years we have known that such intersexual males of a low grade are completely fertile, and the eggs fertilized by them develop normally. It was of course of extreme importance for our work to breed offspring from the higher grades of intersexual males and they were therefore all mated, obviously to the limiting type, which was still male enough to perform the mating. All matings were certainly normal, as every female laid a normal egg sponge, which is only done after a successful mating. From the eggs fertilized by low-grade intersexual males the normal percentage of caterpillars hatched, as in previous years. But from egg batches, fertilized by somewhat higher intersexual males, only a few caterpillars hatched, the rest of the eggs being unfertilized. The numbers were for four cultures 3, 3, 2, 3 caterpillars, the egg batches containing between 100 and 300 eggs. Finally, in the egg batches laid after mating with the highest type of intersexual male, which was still able to mate, not a single egg developed. Now in studying the sex glands of these males we found that in low grade intersexuality they contained normal sperm bundles, but in the higher forms of intersexuality the entire gonad was filled with giant bundles of apyrene spermatozoa. The intermediate forms, which gave a few fertile eggs, were unfortunately not examined. We think it not unsafe to conclude from these facts that apyrene spermatozoa can not induce development, even if they enter the eggs, which, however, also seems improbable.

But if the atypical spermatozoa play no part in fertilization, what is then their function? We think we can derive an answer from some experiments carried on during the winter of 1914–15 on spermatogenesis in vitro,[10] an answer which is in full harmony with the above quoted results of other investigators.

In rearing the sperm follicles of the moth *Samia cecropia* in tissue-cultures, we found that in the fall the follicles taken from the pupæ finished practically all their normal spermatogenesis and a follicle with apyrene spermatozoa never appeared. But in January and February the results were quite different. The fresh material already contained many degenerating follicles. In the tissue cultures only a very few follicles performed the spermiogenesis, most of them dying after repeated unsuccessful trials to undergo the maturation divisions. These testes, however, already contained many apyrene follicles. Later in February some pupæ were kept in the thermostat for a week. In examining their testes they were found filled with sperm bundles, the great majority of them being apyrene.

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[10] A full account of that piece of work has probably appeared meanwhile in the *Arch. f. Zellforschung,* 1916, under the title: "Einige Versuche zur Spermatogenesis in Vitro." A preliminary notice is found in *Proc. Nat. Ac. Sc.,* I., 1915.

In the same experiment it could be shown, further, that the transformation of a sperm-cell into a spermatozoon is caused by the physical condition of the follicle membrane and can be produced artificially to a certain degree. Now one of the main characteristics of the development of the apyrene spermatozoa is the production of caryomerites from the chromosomes and their further degeneration. The same phenomenon has been produced by Conklin[11] in the cleavage cells of *Crepidula* by changing the osmotic conditions of the surroundings. Combining these facts, we reach the conclusion that a definite change in the physical properties of the follicle membrane forces the sperm cells within, physically, to undergo definite atypical changes, which lead to the formation of an apyrene spermium. This process is therefore nothing but the expression of a reaction, necessitated by the physico-chemical properties of the sperm-cell on which the abnormal surroundings act; a reaction produced by abnormal conditions, a teratoma, a lusus naturæ. The typical form of the abnormal sex-cells for a given species is as much necessitated by the specific substratum as the typical form of a plant-gall. The apyrene spermatozoon is a functionless reaction-product.

The results derived from the experiments with intersexual animals are in harmony with this conception derived from the study of tissue cultures. It is well known that the chemical properties of the hemolymph in insects change during metamorphosis in connection with histolysis, and the entire metabolism is put on a different basis, as Weinland[12] proved. In the case of the pupæ of *Samia* it is easy to observe, without going into chemical details, that the blood in old pupæ which produce the atypical spermatozoa has very different properties from those in the young. On the other hand, the work of Steche and Geyer[13] has shown that in the gipsy-moth the chemical characters of the blood are very different in the male and female sex. Hence it might reasonably be expected—tests are going to be made—that in intersexual individuals, where every single character is intermediate to a definite degree between the two sexes, the blood is also different from the normal blood, thus producing in the case of intersexual males those physico-chemical conditions which account for the formation of the apyrene spermatozoa.

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[11] Conklin, E. G., ''Experimental Studies on Nuclear and Cell-division in the Eggs of *Crepidula*,'' *Jour. Ac. Sc.*, 15, 1912.

RICHARD GOLDSCHMIDT
MARINE BIOLOGICAL LABORATORY,
WOODS HOLE, MASS.

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THE SHAPE OF THE STERNUM IN SCORPIONS AS A SYSTEMATIC AND A PHYLOGENETIC CHARACTER

ALEXANDER PETRUNKEVITCH, Ph.D.,
Assistant Professor of Zoology in the Sheffield Scientific School

(From the Osborn Zoological Laboratory of Yale University)

It is generally recognized that the shape of the sternum furnishes one of the important characters for the distinction of families in recent scorpions. The small family Bothriuridæ is the only one in which the sternum is composed of two transverse plates. This family includes seven genera, six of which occur in South America, while the seventh (*Cereophonius*) is an inhabitant of South Australia and contains a single species. The families Scorpionidæ, Vejovidæ, Chærilidæ and Chactidæ have a distinctly "pentagonal" sternum with more or less parallel sides. The Chærilidæ belong exclusively to the old world. The Chactidæ are divided into three subfamilies, the European Euscorpiinæ and the neotropical Megacorminæ and Chactinæ, which have Mexico for their northern limit of distribution. The Vejovidæ are unevenly distributed between the Old World and the New. One of the eight genera composing this family is found on the shores of the Mediterranean (*Iurus,* with a single species *I. dufoureius*), another (*Scorpiops*) with about eight species in East India. Of the remaining six genera, two occur in South America, while the other four belong to the southern and western United States and to Mexico. The family Scorpionidæ, to which some of the largest scorpions belong, has representatives from various countries and regions. It is usually divided into five subfamilies. Of these the Urodacinæ are Australian; the Scorpioninæ Asiatic and African; the Hemiscorpioninæ Asiatic; the

Ischnurinæ African, Asiatic and neotropical (*Opisthacanthus elatus* is the single neotropical species); the Diplocentrinæ neotropical and Asiatic (a single genus with two species from eastern Asia).

All scorpions belonging to the large family Buthidæ have a distinctly "triangular" sternum with converging sides and truncated apex. The family is naturally divided into two large subfamilies. Of these the Buthinæ may be regarded as belonging to the Old World, since of its 14 genera a single genus and species (*Ananteris balzani*) is found in South America. The subfamily Centrurinæ includes four genera. The genus *Isometrus* is characteristic of the Old World but its commonest species, *I. maculatus,* is cosmopolitan and occurs in Florida, Hawaiian Islands, South America, etc. *Zabius* is South American. *Titius* is neotropical, although one species, *T. floridanus,* occurs in southern Florida. The largest genus, *Centrurus,* is represented by some of the commonest species in the southern United States and the subtropical and tropical America.

Let us fix our attention for a moment on the distribution and characters of two genera of scorpions common to the United States. One is *Vejovis* (of the family Vejovidæ) and is represented in this country by six species; the other is *Centrurus* (of the family Buthidæ) and is represented by seven species. *Vejovis* belongs more to the southwest and west. It is distributed through California, Nevada, Utah, Arizona, New Mexico and Texas and extending northward into Idaho and Nebraska. A single species, *V. carolinus,* is found in the southeast. It occurs as far north as South Carolina and spreads southward to the Gulf states and Texas. Except possibly this species, the other species of *Vejovis* occur also in Mexico where the genus is represented by four additional species which do not occur in the United States. I have besides, in my private collection, a new species of *Vejovis* from Terra del Fuego. *Centrurus* belongs more to the southeast. A single species (*C. exilicauda*) occurs in

California. *C. nigrescens,* a variety of the more southern *C. gracilis,* has been reported from Texas. Four species occur in Florida, but of these *C. gracilis* and *C. margaritatus* belong to a more southern fauna, the latter being the most common scorpion of Mexico and Central America. One species, *C. infamatus,* has practically the same distribution as *Vejovis carolinus,* spreading northward into South Carolina and southward into Texas and northern Mexico. *C. infamatus* is the common scorpion of the southeast and south. Several other species of *Centrurus* are characteristic of tropical North and South America.

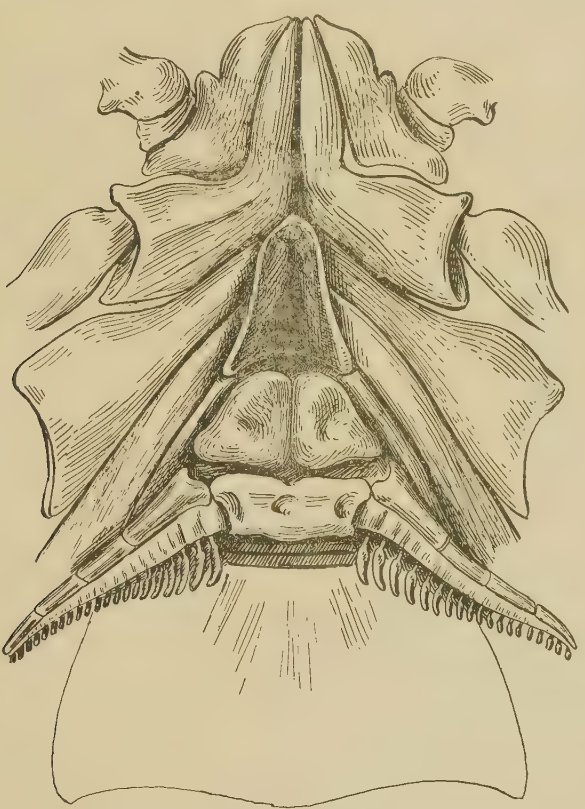

FIG. 1. Sternum, genital opercula, combs and coxæ of an adult *Centrurus insulanus* Thorell from Jamaica, W. I. The sternum is "triangular" with converging sides, but still shows traces of its original, pentagonal shape.

Vejovis carolinus is a small scorpion, its length not exceeding 34 mm. The width of the carapace at the posterior edge is slightly less than the length. The center of the eye tubercle is about ⅖ of the entire length of the carapace from its anterior edge. The sternum is pentagonal; the comb has 13–14 teeth; the central and inner rows of plates in the comb are beadlike. The fingers are rather short, being either as long as or only slightly longer than the hand.

Centrurus infamatus is usually about 45 mm. long, but large specimens measure up to 70 mm. The width of the carapace at the posterior edge is equal to or even slightly exceeds its length. The center of the eye tubercle is about ⅖ of the entire length of the carapace from its anterior edge. The sternum is triangular. The comb usually has 18–19 teeth, although their number may reach 25. The central rows of plates is not beadlike, but composed of five plates the limits between which are difficult to ascertain. The inner row is beadlike. The fingers are rather long, being more than 1½ times as long as the hand.

Let us now consider the Palæozoic scorpions. The sternum of the Silurian *Proscorpius osborni* Whitfield is unfortunately not preserved. Its nearest European relative, the Silurian scorpion *Palæophonus hunteri* Peach, has, according to Pocock, a pentagonal sternum. The sternum of the carboniferous scorpions is fairly well preserved in many specimens. The family Isobuthidæ differs from all other fossil and recent scorpions in the position of the fourth pair of coxæ which abut against the genital opercula. The sternum is either triangular (*Palæobuthus*), rhomboidal (*Isobuthus*) or oval (*Eobuthus*). The family Cyclophthalmidæ has a "pear"-shaped sternum, the family Eoscorpionidæ a distinctly pentagonal one with parallel sides. If the pear-shaped and rhomboidal impressions of sterna do not owe their shape to poor preservation or displacements, then these types of sterna must have disappeared completely, as has the type of triangular sternum found in Isobuthidæ. Of preserved fossils there remain then only the Silurian Palæophonidæ and the carboniferous Eoscorpionidæ having a sternum and arrangement of coxæ similar to that in recent scorpions. But the Silurian scorpions possessed other characters of their own and have either disappeared completely or perhaps have changed gradually into carboniferous forms. In the absence of Mesozoic fossils any attempt to trace relationships between recent and Palæozoic scorpions can be only conjectural. Thus in my "Monograph of Palæozoic Arachnida" I arrived at the conclusion (p. 26) that "the family Eoscorpionidæ shows many relations to the recent Scorpionidæ and Vejovidæ and represents probably two or three families thrown together for lack of distinctive characters." In formulating this opinion I was guided chiefly by the shape of the sternum, in several cases remarkably well preserved. Since that time I have made an observation, insignificant in itself, but one which affords an insight into the past history of recent scorpions possessing a triangular sternum and suggests a closer relationship between the Eoscorpionidæ and the Centrurinæ than between the former and the Vejovidæ. This observation was made by pure chance. While studying the problem of segmentation in Arthropods, I was examining a frontal section through a recently born *Centrurus insulanus* from Jamaica (Fig. 2). To my amazement the sternum proved to be beautifully pentagonal. An error of identification was excluded. I myself collected the material and preserved the adult females with the young carried on their back. Yet if objection should be raised, a final proof is offered by the fact that late embryos, too, have a pentagonal sternum and such embryos are easily obtainable from adult, gravid females. (Scorpions are without exception viviparous.) The young of *Centrurus infamatus* also have a pentagonal sternum, as have probably the young of all other species of the genus *Centrurus*. It is strange that no one has noticed this before, since there must be dozens of specimens in every museum. I find an interesting confirmation of my observation in Fig. 10 of McClendon's paper on the nervous system of *Centrurus infamatus*.[1] The adults of his material were identified for him by no less an authority than Kraepelin, yet in the figure in question McClendon draws a distinctly pentagonal sternum in a surface view of a late embryo. The case is the more interesting because McClendon himself is unaware of the value of his observation, nor is there the slightest reference to it in the text. He simply drew the sternum as he saw it, without so much as mentioning it. An examination of the adults of *Centrurus insulanus* (Fig. 1) as well as of other species of *Cen-*

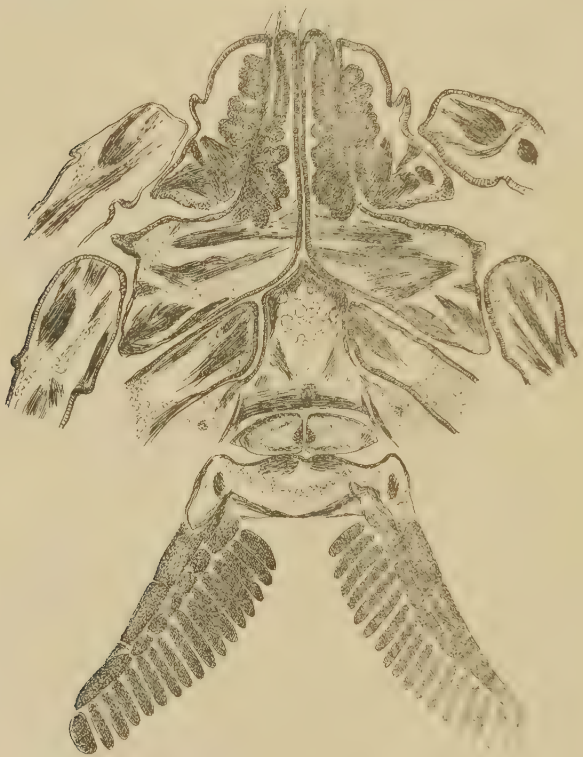

FIG. 2. Frontal section parallel to the ventral surface showing sternum, genital opercula, combs and coxæ in a very young *Centrurus insulanus*. The sternum is distinctly pentagonal, with parallel sides.

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[1] *Biol. Bull.*, Vol. 8, 1904, p. 51.

trurus, shows that their sternum retains throughout life traces of its origin from a pentagonal prototype. Only the triangular apex is comparatively small and more or less hidden between the coxæ, and the sides of the sternum are strongly convergent, not parallel. The sternum in adults of various species of Buthinæ of the Old World shows also a small triangular apex hidden between the coxæ, and in the absence of material I venture the prediction that the embryos will be proved to possess a pentagonal sternum.

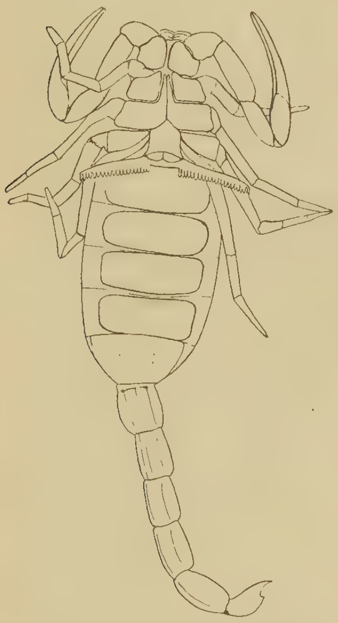

Fig. 3. A very young *Centrurus infamatus* (C. l. Koch) = (*carolinianus* Palisot de Beauvais) drawn with the aid of the Abbe apparatus. The sternum is pentagonal. The hand has much longer fingers than would be the case in an adult of the same species. Abdomen distended by yolk.

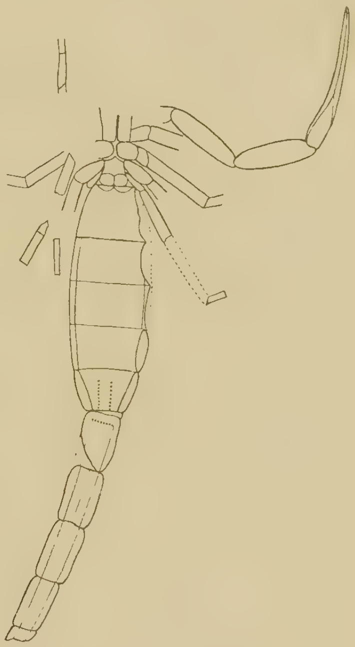

Fig. 4. *Eoscorpius typicus* Petrunkevitch, from Mason Creek, Illinois. Specimen No. 37986 of the U. S. N. M. (Fig. 6 of the Monograph.)

Keeping this in view, let us now compare *Centrurus infamatus* with *Eoscorpius typicus* of the Pennsylvanic from Mason Creek, Illinois, a rather well-preserved Palæozoic scorpion of approximately the same size. Fig. 4

is taken from my monograph and represents the holotype of *Eoscorpius typicus* as it appears on the reverse. Fig. 3 is a careful drawing of a very young *Centrurus infamatus* made with the aid of an Abbe apparatus. This figure is much more enlarged than is Fig. 4, to facilitate comparison. The shape of the abdomen, as is well known, is the least constant character and we will leave it out of consideration. To avoid criticism, however, I would state that the great distension of the abdomen in the young *Centrurus* is due to the presence of embryonic yolk. When this disappears, the abdomen becomes much thinner. In older specimens the distension of the abdomen is frequently due to the growing embryos. Yet, in dissecting what I supposed to be gravid females, I was surprised to find no embryos in them and only small ovaries. The fact is that the distension of the abdomen is also often due to the condition of the liver.

The carapace has a fairly constant shape and is similar in both scorpions. For figure and measurements I refer to my monograph. I do not reproduce them here because the carapace of *Vejovis*, except for its size, is also of similar shape. The rows of granules on the caudal segments are not sufficiently well preserved in the fossil specimens to allow of a conclusion as to their exact number. On the other hand, the transverse row of granules at the anterior end of the first caudal segment, present in *Eoscorpius* and wanting in the adult *Centrurus*, is clearly defined in the young. The most interesting character is represented in the hand with its fingers. As a rule the ratio between the length of the fingers and that of the hand is a fairly constant one for each species. In the holotype of *Eoscorpius typicus* it is approximately 2:1; in the adult *Centrurus infamatus* it is 1.6:1, but in the late embryos and just born young it also approximates 2:1. With other words, *Centrurus* developed from an ancestor with relatively longer fingers and the trend of evolution was toward reduction in their length.

One character presents a difficulty. This is the comb.

Specimen No. 37987 of the U. S. Nat. Mus. of *Eoscorpius* shows a comb which is very broad at the base, and has, apparently, a single median plate, a beadlike inner row and 19 teeth. I identified this specimen as *Eoscorpius typicus* for the reason that "the general appearance of the specimen, the shape "of the tergites, especially of the seventh," strongly resembled the holotype. The specimen is incomplete and about twice as large as the holotype. Perhaps No. 37987 is after all of a different species. The shape of the comb in recent species of *Centrurus* is not always the same as in *infamatus*. The comb in *C. junceus* and *C. agamemnon* is twice as wide at the base as in the middle.

Taking all characters and the geographic distribution into account, we can not fail to notice the greater similarity between the young of *Centrurus infamatus* and *Eoscorpius typicus* than between the latter species and *Vejovis*. What advantage, if any, *Centrurus* has derived from the shortening of the fingers and the change in the shape of the sternum, is a totally different question which may possibly be answered by studying the functions and uses of these organs in recent species.

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October, 1916

ON THE BLASTOLYTIC ORIGIN OF THE 'INDEPENDENT' LENSES OF SOME TERATOPHTHALMIC EMBRYOS AND ITS SIGNIFICANCE FOR THE NORMAL DEVELOPMENT OF THE LENS IN VERTEBRATES[1]

E. I. WERBER
Osborn Zoölogical Laboratory, Yale University

TWO TEXT FIGURES AND TWO PLATES

The problem of the development and differentiation—dependent or independent—of the lens of the vertebrate eye has in recent years formed the subject of many interesting investigations.

In 1901 Spemann attempted its solution by experiments in which the anlage of an optic cup in the neurula-stage of Rana fusca was mechanically destroyed. From the failure of many larvae to develop a lens on the side operated upon he concluded that the lens of the vertebrate eye depends for its development upon a contact stimulus from the optic cup. Herbst ('01) concluded from the fact that in higher vertebrates the development of the lens never begins before the optic vesicle has come into contact with the ectoderm from which it arises that the latter is essential and that the development of the lens is to be regarded as a 'specific' (chemical?) 'thigmomorphosis.' As evidence for the justification of this hypothesis he adduced the cyclopean monsters, i.e., embryos with a single median eye. In such embryos, he reasoned, no lenses develop on the sides of the head, that is, in the usual position of the eyes, because the ectoderm of these areas, owing to the absence of lateral optic vesicles, does not receive the specific thigmomorphotic stimulus necessary for its differentiation into lenses. On the other hand, he concluded, since a lens does develop in association with the

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[1] Aided by a grant from the Carnegie Institution of Washington.

median eye from ectoderm which normally does not give rise to a lens, it is evident that the heterotopic (cyclopean) optic vesicle furnishes the formative stimulus for its differentiation from indifferent ectoderm.

Soon, however, the validity of this exceedingly suggestive hypothesis, became questionable, for Mencl ('03) described two lateral lenses in an anophthalmic head (without any traces of optic cups) of an anadidymus in Salmo salar. From these findings he concluded that no stimulus from the optic cup was necessary for the differentiation of the lens. More recently Mencl ('08) has recorded free lenses in several anophthalmic Salmo embryos.

In the meantime the problem had again been attacked experimentally by Barfurth ('02) W. H. Lewis ('04), King ('05), Le Cron ('07) and Spemann ('07). The evidence which Barfurth was able to bring forward for the 'dependent' differentiation of the lens in the chick was rather inconclusive. Of a very decisive character, however, seemed to be the results which Lewis obtained in his experiments on Rana palustris. He removed the optic vesicle of tadpoles with the least possible injury to the overlying ectoderm which was raised from it and reflected forward. No lenses developed in such embryos on the side operated on and Lewis' conclusions fully confirm Herbst's views. Only a year later, however, King experimenting on embryos of the same species by destroying the optic vesicle with a heated needle, was able to show that lens buds developed on the eyeless side of the embryo which might perhaps have fully differentiated into lenses, had the embryos been kept alive for a sufficient length of time. This remarkable discrepancy of results increased from now on with practically every attempt at the solution of the problem. Le Cron, experimenting on Amblystoma was able to confirm Lewis' important findings, but Spemann ('07) found that in Rana esculenta a lens may be formed without any stimulus from an optic vesicle or cup. In the next year Mencl was able to confirm the observations he had recorded in 1903, and in 1909 Stockard employing a chemical method, succeeded in producing in Fundulus experimentally many teratophthalmic monsters in which the free lens was of rather frequent occurrence. From his observations which strongly corroborate Mencl's statements, Stockard concluded that the lens is capable of development without any stimulus from the optic cup.

Mencl's and Stockard's results and their conclusions seemed to be very convincing, and, on the whole, the major evidence seemed to be in favor of the independent origin of the lens by 'self-differentiation.' The climax of the difficulty, however, had not yet been reached, for, in 1912 Spemann reported on the basis of very extensive experiments that the same methods of operation on the same stages of frog larvae of two species belonging to the same genus yielded conflicting results, the one species being capable of forming lenses without the stimulus of an optic cup, while in the other species lenses were not formed if the optic vesicle had been removed.

What is the cause of this remarkable incongruity of apparently correct observations? Why is Amblystoma incapable of forming a lens independently of the optic cup, while in Rana esculenta this ability apparently exists? Why is Rana palustris capable of differentiating a lens without the contact stimulus from an optic cup, when the optic vesicle is destroyed by a heated needle and incapable of doing so when the latter is removed by cutting? Why in cyclopean Fundulus embryos does the single median optic vesicle stimulate by contact the formation of a lens from ectoderm which would normally not differentiate into a lens, while in other teratophthalmic, and particularly, anophthalmic embryos of the same species free lenses frequently occur without any trace of an optic cup? And, finally, why should two species of the same genus differ so widely in their morphogenetic potentiality, the one (Rana esculenta) being capable of forming a lens when the optic vesicle has been removed, while in the other (Rana fusca) this apparently does not occur, after the same operation performed at the same larval stage?

The answer to these perplexing questions, I think, is that we are here dealing with an apparently morphological problem for whose solution neither our morphological methods of investigation nor mechanical methods of experimentation seem to be fully adequate. The problem is, as I hope to make clear in the following, pre-eminently a biochemical one, as Lewis has suggested already in 1904.

During the summers of 1914 and 1915 I performed a large number of experiments on fertilized eggs of Fundulus heteroclitus by exposing the latter in early cleavage stages to some toxic products of pathologic metabolism in order to test the hypothesis that these products may underlie the 'spontaneous' origin of monsters. Among the many monsters thus produced those with terata of the eyes were found to be of most frequent occurrence. In some of the latter free lenses were found. The location of such 'independent' lenses is strikingly variable. Thus, for instance, a cyclopean embryo was once found, which had besides the lens of the median eye also a free lens, normal in size and structure, in a lateral position (Werber '15 a, fig. 28, p. 551). In a symmetrically monophthalmic embryos a lens of approximately normal size may sometimes be found on the side lacking the eye, or several small lenses may be met with on various parts of the head in a lateral or anterior position. In such and in anophthalmic embryos one or more small lenses are sometimes found on the anterior tip of the head, while in one asymmetrically monophthalmic embryo three small lenses were found on the maxilla (Werber '16 b, figs. 78 and 79). In many instances small free lenses were also observed posterior to one of the eyes in deformed embryos possessing two eyes in the usual lateral position.

The perfect analogy between these observations and those of Mencl's (l.c.) and Stockard's (l.c.) is evident. For in all these cases lenses have developed, without the contact stimulus from an optic cup, from indifferent ectoderm which would normally not have given rise to such structures. If Mencl's and Stockard's interpretation according to which these lenses are to be considered as products of self-differentiation were justified it would necessarily apply also to the free lenses which I have recorded. Yet, is the evidence of Mencl and Stockard conclusive? Will it stand the test of critical examination? I believe that I shall be able to show in the following that these questions cannot be answered in the affirmative.

It is very significant that free lenses are found only in teratophthalmic embryos. No case has yet been recorded of a free lens in the presence of two normal, perfect eyes, while such a lens may occasionally be found in the presence of both eyes if the latter exhibit some structural imperfections. And in such cases of monophthalmia asymmetrica, where the single lateral eye is in perfect structural harmony with a normal eye, a free lens, if found, is always on the side lacking the eye. Briefly it may be said that free lenses do not occur where there is no eye defect. This rule is so constant that some correlation between the eye defect and the 'independent' lens must necessarily be assumed. Granting this (and the evidence to prove just this point, is, indeed, overwhelming) it might be expected that if it were possible to determine the nature of the defect, i.e., the mechanism which causes it, we would at the same time obtain a very definite clue towards the genesis of the free lens.

I have attempted an analysis of the nature of the defect which leads to teratophthalmia, and, on the basis of very definite and constantly increasing evidence, have arrived at the conclusion that we are here dealing with a destructive process of dissociation of parts of the blastoderm. The nature of this process, which I have termed blastolysis, has been very briefly outlined in two of my previous publications (Werber '15 b and '16 a) while a more complete treatment of the subject will be found in a paper now in press (Werber '16 b) to which the reader is particularly referred.

For the sake of clearness in the following presentation it may be stated, however, that blastolysis either destroys part or all of the germ's substance or it may dissociate and disperse fragments of the latter.

A careful study of the morphology of teratophthalmic embryos has yielded unmistakable evidence that all of them are due to a defect of blastolytic nature in the anterior region of the head. In other words, the formation of terata of the eye comes about through destruction of parts intermediate to the earliest anlagen of the eyes or of parts of the latter. The most striking evidence for this blastolytic process is dispersion of fragments of ophthalmoblastic material which sometimes develop into fragments of an optic cup. I have observed several such instances and have always found that the relation between such a teratoma and the terata of the eyes in the same embryo is clearly one of syngenesis. In several of such embryos free lenses were found which were located far away from an eye or optic cup fragment.

These observations which fully agree with Stockard's ('09, '10) and Mencl's ('08) findings,[2] and the fact mentioned above that free lenses occur only in teratophthalmic embryos suggested that the origin of such lenses is in some causal connection with blastolysis. The dispersion of ophthalmoblastic material and the simultaneous presence of free lenses in some teratophthalmic embryos suggested strongly that the origin of the latter depends upon the former. While examining sections of many teratophthalmic embryos during the winter of 1915 this hypothesis forced itself upon me by the sheer weight of the remarkable concurrence of facts, which could not, by any means, be considered, as fortuitous. For, why do free lenses arise only in such cases where the eyes are defective as a result of blastolysis, that is, where ophthalmoblastic substance has been partly destroyed, and partly dissociated or dispersed?

These considerations have, finally, led me to the following theoretical conclusion which I regard as a possible basis for more refined experiments towards the solution of the lens-problem.

It could be imagined, I thought, that just as in normal development two lateral lenses arise as the result of an apparent contact stimulus from two lateral optic cups and just as in the cyclopean eye one median lens arises as a result also of a contact stimulus of one median eye, the free lenses of teratophthalmic embryos could arise from indifferent ectoderm owing to a stimulus from a fragment of ophthalmoblastic material too small to differentiate in the state of its isolation into a morphologically discernible structure. This stimulus is, as Herbst (l.c.) has pointed out, not a contact stimulus only, but very probably a specific thigmomorphosis, the mechanism of which I am inclined to consider as similar to (or possibly identical with?) an enzyme reaction. In other words it might be imagined that ophthalmoblastic substance (and most likely potential retina) when in contact with epidermis acts as a catalyzer for the chemical reactions necessary for the transformation of the latter into a lens.

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[2] Cf. Stockard ('09, fig. 45, p. 317 and '10, figs. 6 and 8, p. 403 and figs. 22, 23, 24, p. 409) and Mencl ('08, plate xx, figs. 5, 6, 7, and 8).

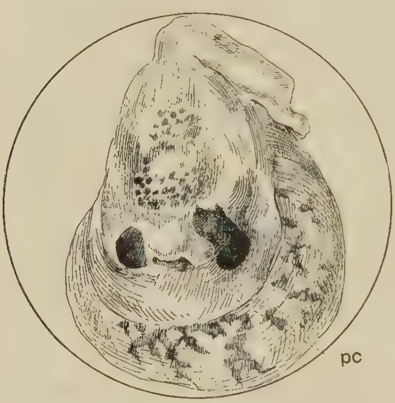
Very strong support is lent to these conclusions by the results of some more recent experiments in which I have employed a modification of one of my methods. By employing weak solutions of acetone and long exposures I expected that embryos might thus result in which less destruction and more dissociation would be noted. And such was actually found to be the case.

While some of the eggs so treated have given rise to synophthalmic or monophthalmic monsters, others have developed into embryos with two large, grotesquely protruding eyes in the usual lateral position in the head (fig. 2). Some of the embryos from these experiments possess two ill-formed eyes of medium size, one of which may often be far protruding, or in some cases one or both eyes may already on macroscopic examination exhibit evidence of dissociation, the pigment layer being either partly duplicated (fig. 1), or fragments of it being observable between or behind the eyes. Examination of sections shows that these eyes are elongate and ovoid in shape and it seems clear that during their formation some forces must have been acting which tended to disintegrate and dissociate the anlage from which they have developed (blastolysis).

A detailed description of the microscopic anatomy of such 'blastolytic eyes' is reserved for another publication, soon to follow. For the present it will suffice to state that in such embryos a number of free lenses of various sizes often are found in the head region on examination in toto, while each one of the eyes may possess a lens of its own in contact with the optic cup. Examination of sections of some of these embryos, however, discloses a most surprising multitude of small lentoids anterior to the eyes.

A brief description of sections through the anterior part of the head of one such embryo may now follow. Already in toto (fig. 1) it is seen that the eyes are greatly deformed, only their pigmented parts being discernible. The right eye appears to be rather small, while the left eye is large and protruding and exhibits striking evidence of dissociation. Transverse sections (6μ in thickness) show the following conditions:

Already in the second section there can be seen at the base of the forebrain on both sides a number of small lentoid bodies, which if followed in succeeding sections are found to form an almost continuous mass. In the same section one still smaller lentoid is seen on the periphery of the integument, while in further sections nearly all of the strata of some parts of the epidermis seem to be transformed into such lentoids. At this level (48μ from the tip of the head, figure 3)[3] there is seen at the base of the brain a fairly large lentoid with a number of smaller ones near by. All lentoids so far recorded in this description are in a stage of differentiation transitional between the epithelium of the lens-bud and the fibrillae of the fully developed lens. Follow-



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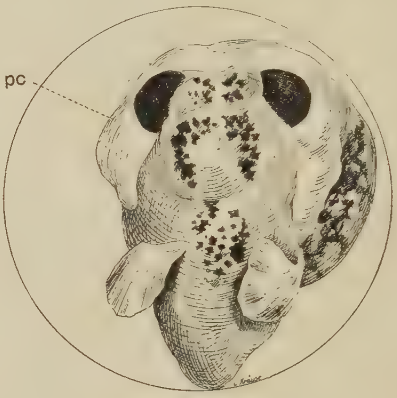

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Fig. 1 Teratophthalmic embryo, twenty-nine days old, from acetone solution (20 cc. gram molecular in 50 cc. sea water). Exposure one hundred and forty-four hours, solution renewed every day.

Fig. 2. Teratophthalmic embryo, twenty-three days old, from the same experiment as the embryo in figure 1.

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[3] It is a pleasure to acknowledge my indebtedness to Professor Petrunkevitch for his kind assistance in making the photomicrographs.

ing the sections caudalwards, we find in the tenth section (60μ from the tip of the head) the beginning of the lens of the right eye. In the section preceding it is seen the capsule of the lens on two sides of which several small lentoids are noted. The lentoid mass at the base of the brain is no longer seen at this level; only a few very small lentoids can be observed in this position in the eleventh and twelfth sections. In the latter there appears also the first indication of the pigment layer of the left eye. Two sections more caudalwards the optic cup of the right eye begins to come into view. At this level the optic cup of the left eye can already be clearly distinguished. Its pigment layer is of a dark brown hue instead of a solid black, which is due to the fact that the pigment cells are considerably dissociated. The layer of rods and cones, while not fully differentiated, is, however, clearly discernible. All the other layers of the retina are spread out far beyond the limits of the optic cup into a continuous mass which reaches the lens of the other eye and goes all around the unusually small oral cavity. From the epithelium of the latter there arise several small lentoids which are in contact with this blastolyzed retina. Four sections more caudalwards the mouth disappears, its place being taken up entirely by six small lentoids which are partly in connection with each other through lens fibers. In this section (fig. 4) and in three more succeeding sections the dissociated pigment layer of the left eye presents a strange appearance. It bulges out on one side of the optic cup to form a very large pocket into which a part of the retina dips. This pigment 'pocket' corresponds to a part of the apparent duplicature of the pigment layer of the left eye seen in toto in figure 1. The left eye possesses no lens but its blastolyzed retina surrounds the six oral lentoids spoken of above, which in more posterior sections increase in number and form a rather dense cluster. A number of small lentoids can be observed also in the epidermis ventrally from the eyes.

The optic cup of the right eye is, as compared with its large lens, strikingly small and its tissues, while dissociated, are much less so than those of the other eye. However, the blastolytic dissociation of this eye is plainly discernible in posterior sections.

A very remarkable feature is presented by this optic cup in the twenty-eighth and twenty-ninth sections (fig. 5). Here a small body is found which has the same staining reaction as the lens of this eye and all the lentoids observed in the embryo. It is situated in proximity to the layer of rods and cones and is surrounded by practically all other layers of the retina. On first examination the impression was gained that we are here dealing with an artifact, namely a lens fragment carried in by the knife during sectioning. This suspicion, however, proved to be unfounded for the following reasons. First, the structure appears in two sections in exactly the same position, and it is larger in the second section containing it, the part observed in the preceding section being its tip. Secondly, if it were an artifact, it would necessarily have to overlie a corresponding part of the retina, which is not the case, for on deep focusing on the first part (contained in section 28) nothing can be observed below the section of this structure, while a deep focus on its second part (section 29) discloses only pigment cells, with which it is surrounded on all sides but one. The morphogenesis of this lentoid is, of course, uncertain. But it seems probable that we are here dealing with a 'retinal lentoid' such as was in many instances observed by Fischel ('02) after mechanical injury of the retina. In this case our retinal lentoid would have to be regarded as a case of embryonic heteromorphosis induced by chemical alteration of the environment.

More evidence for blastolysis is presented by the dissociated epidermis,[4] and the unusually distorted shape of the brain of this embryo as well as by the significant fact that in posterior sections of the eyes there appear dorsally from the latter two more rudimentary optic cups, the retina of which (rods and cones) while not fully differentiated, yet is clearly discernible. These optic cups have not been pushed out by the brain, but each one forms a part of its dorso-lateral wall almost exactly where one would expect the optic lobes, which latter, owing to the distortions of the brain, can nowhere be recognized. This indicates that the ophthalmoblastic material of each side has been fragmented by blastolytic action of the modified environment into two parts, one of which was capable of differentiating almost as much as in normal development, while the other attained only a rather low degree of differentiation.

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[4] The embryo was alive when fixed. All my observations on teratological material were made only on embryos alive at the time of fixation.

The longitudinal axes of both eye bulbs are dorso-ventral instead of transverse as they should be in the fish. This deviation in their position in relation to the brain is, undoubtedly, also due to blastolytic dissociation and subsequent readjustment of parts. Owing to this dissociation the optic anlagen have during their progress towards the unusual position which they finally have come to occupy, left fragments of optic material in their trail, and have in this way stimulated a number of lentoids to arise, which can be found in a number of sections, most prominently, on the right side, between the last part of the eye and the rudimentary optic cup of the same side (fig. 6) At this point (the region of fragmentation of one original eye anlage into two) a loose mass of apparently disintegrated tissue, only the nuclei of which have for the most part remained, can in a number of sections be observed to extend all the way from the rudimentary optic cup with which it is continuous into the oral cavity. The latter is at this point defective, a part of its cartilaginous roof lacking. Careful examination of sections shows that the nuclei of the disintegrated tissue mass are mainly of two sizes. The impression is gained that these nuclei are the remnants of potential retinal cells, the large ones possibly corresponding to the cells of the ganglionic layer while the small ones to those of one of the molecular layers. If we imagine that the disintegrated tissue mass was fragmented and dispersed from the earliest primordium of the eye of this side, it is conceivable that many of its cells were yet capable of farther development and have made some steps in differentiation. To the latter may be due the differences between the nuclei of this 'syncytium,' corresponding to differences between the various layers of the retina. At any rate, the tissue mass in question suggests forcibly that we are here dealing with a retinal fragment, which was largely disintegrated after some differentiation. It is only necessary to compare it with sections through the anterior part of the eyes where areas of such disintegration and intermingling of 'naked' nuclei of the same two types are also found, to disarm any skepticism on this point.

If, however, it is granted that the tissue mass in question is retina, the origin of the lentoids of this region as well as in the most anterior part of the embryo can no longer be doubted as due to contact of ectoderm with dispersed particles of ophthalmoblastic substance. Blastolysis has in this way affected also the brain, which besides its unusual distortions also exhibits distinct traces of disintegration. Part of the cartilaginous roof of the mouth in this region is also lacking and it is this part that is filled with disintegrated retina. If the sections be followed caudalwards the roof of the oral cavity is observed to appear gradually more and more complete until a section is reached where the defect no longer exists. At this level, no more disintegrated retina is found in the mouth, but imbedded in the oral epithelium a number of lentoids can be observed in six sections (fig. 7). Beyond this level the epithelium of the corresponding part of the oral cavity consists in several sections of large vesicular cells which look decidedly as if they were in a stage intermediate between the epithelial and early lentoid. Of all the evidence for the blastolytic origin of the lentoids of this embryo presented so far, the lentoids of the oral cavity form, I believe, the most striking if not the most important, part. For here the lentogenic reaction (as I would term it) of ectodermal epithelium to optic cup substance is demonstrated in a manner, which would seem to leave no room for reasonable doubt.

Not less obvious is the conclusion to which these observations would seem to lead. The free lenses of teratophthalmic embryos recorded by Stockard, Mencl and myself, are not independent in their differentiation, but due to a specific (catalytic?) reaction of dispersed fragments of optic cup substance on the ectoderm with which they may chance to come in contact.

Mencl's and Stockard's observations regarding the free lenses in teratophthalmic embryos which seemed to form such important evidence against Herbst's theory of the developmental correlation between optic cup and lens can now, in the light of our findings, be fully harmonized with it.

Of the 'independent' lenses recorded in the experiments of other authors, those described by King (l.c.) can easily be accounted for, when it is considered that the method employed in her experiments contains an uncontrollable source of error. For it is easy to see that of the optic vesicles of frog larvae which she destroyed, some particles might have been dispersed and come into permanent contact with the overlying epidermis in which in this manner the 'lentogenic reaction' was induced. Provided that this conclusion is correct (and I think it is highly probable that it is) it is on the other hand just as easy to understand why for the same frog species in the experiments of Lewis (l.c.) in which a more reliable method was employed, the recorded results were the opposite to those of King's. By skillful operations, Lewis removed the optic vesicle in such a manner that no fragments of it came into contact with overlying ectoderm. Therefore the 'lentogenic reaction' was never induced in the latter on the side operated upon. The same would seem to hold good for the experiments on Amblystoma performed by Le Cron (l.c.) under Lewis' direction. It follows from these considerations that the free lens buds recorded by King are in their origin similar to those of blastolytic origin in teratophthalmic embryos, the dissociation or dispersion of optic vesicle substance (histolysis) having been induced by mechanical force.

Greater difficulty is presented to our interpretation by the contradicting results which Spemann ('12) has obtained in two frog species of the same genus. However, this difficulty is not insurmountable, for I am firmly convinced that the apparent discrepancies in the effect of the same operations in species of the same genus are not due to primary differences in morphogenetic potentiality, but rather to some secondary factor, which, however, in these minutely delicate operations becomes of great importance inasmuch as it may present a source of unavoidable error similar to that which I have assumed as present in King's experiments. Spemann ('12) himself has pointed out some technical difficulties which may affect the accuracy of experimentation. Thus, for instance, he has observed that the consistency of the embryonic tissues varies with the different frog species, the substance of embryos of some species being more rigid and thus easier to operate on, while in other species it may be very sticky and offer great difficulty to mechanical separation of ectodermal epithelium from underlying cell layers. I am therefore inclined to conclude that owing to this difficulty in many experiments in which Spemann attempted the complete elimination of an eye vesicle, some fragments of the latter were left attached to the epidermis, from which they eventually stimulated the formation of lenses. Such fragments may be too minute for detection with the aid of the binocular microscope and too small to differentiate histologically; yet they may be capable of furnishing the stimulus for the lentogenic reaction, for which perhaps only a very few potential retinal cells may suffice. This might account for the development of the independent lenses in such experiments of Spemann where an optic vesicle has been removed, while in the species in which no such lenses have developed, although the same operation had been performed, it is evidently easier to separate with perfect accuracy the optic vesicle from the overlying epidermis and to remove it in such a manner that no traces of it are left. In view of Spemann's well known skill in such minute operations and his scrupulous care, we may, I think, expect that he will sooner or later be able to confirm this interpretation of the divergence in the results of these as well as other experiments (transplantation of epidermis from a posterior region of the embryo onto an eye vesicle from which the epidermis has been removed—Spemann, '12).

If it were (and perhaps it some day will be) possible to control the difficulties spoken of, it would most likely be found that there are no exceptions to the rule that the lens of the vertebrate eye cannot originate independently of a stimulus from optic cup substance. The difficulty presented to the lens-problem by Mencl's and Stockard's observations may now be considered as no longer existing. For the 'independent' lenses recorded by these authors in teratophthalmic embryos are due solely to 'infection' of the ectoderm with particles of blastolyzed optic cup substance. In a similar manner we may perhaps yet succeed in obtaining identical results in those amphibian species which at present form the vexing exception to the rule of the dependent differentiation of the lens. The chemical method has some advantages over the most refined mechanical method of experimentation. Might it not perhaps, if employed on amphibian eggs, help to harmonize the contradicting results of the experiments with mechanical methods? Judging from results of some preliminary experiments[5] which I have performed with acetone solutions on frog eggs, I am inclined to think that blastolytic teratophthalmia can in these eggs also be produced. And it seems safe to expect that free lenses which so often occur in teratophthalmia,. may also be found in such teratophthalmic amphibian embryos. I venture to predict that results similar to those in the embryo here described may be obtained, if weak solutions of acetone and long exposures be employed.

Another possibility for the origin of free lenses in teratophthalmic embryos is suggested by the retinal lentoid of our embryo (fig. 5). I regard the latter as a tissue heteromorphosis due to a local chemical injury of the potential retina. This injury may not be strong enough to destroy the part affected, but rather it may consist in so decreasing by chemical alteration its chemomorphic potentiality as to cause its ultimate differentiation into a structure less complex than it was 'destined' to form. This embryonic heteromorphosis has its analogue in the regenerative heteromorphosis presented by Fischel's ('02) retinal lentoids, the differentiation of which resulted from mechanical injury of parts of the retina. It would seem not improbable that in Fischel's experiments, too, chemical alteration which resulted from the mechanical destruction has so decreased the chemomorphic capability of the injured parts of the retina that the potency for hypotypical regeneration (lentoids) only has remained. However this may be, it is evident that optic cup substance may under certain circumstances develop into bodies of lens-like structure. If we now recall the fact that eye terata result from blastolytic injury of the potential optic cup, it is easy to imagine that some free lentoids found in such embryos may owe their origin to heteromorphosis from dispersed optic cup fragments. However, it seems doubtful whether fully differentiated free lenses of large size originate from this source.

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[5] Not published.

While, of course, this possibility cannot be entirely disregarded, the evidence points to the conclusion that the fully differentiated free lenses found in some teratophthalmic embryos are due to a chemical stimulus of blastolyzed potential optic cup substance on any part of the ectoderm with which it may chance to come into contact. It is quite possible that this stimulus (the lentogenic reaction) is in the nature of a catalytic reaction such as was assumed by Herbst ('01) to underlie the differentiation of the secondary sexual characters owing to products of internal secretion of the sex glands.

Reactions of this nature (autocatalysis) have in recent years been assumed by J. Loeb ('02, '09), Robertson ('08) and Hagedoorn ('11) and others to underlie the mechanisms of development, growth and inheritance. According to Hagedoorn the hereditary (genetic) factors are to be regarded as autocatalytic substances. A similar view has been advanced recently also by Goldschmidt ('16) who pointed out a quantitative analogy between genetic factors and enzyme reactions in the inheritance of wing pigmentation by various moth hybrids of a known gametic constitution.

The significance of these views is obvious. For, if their correctness should be established, we should gain a very deep insight into the governing forces of development. The evidence will be the more valuable if it is furnished by both genetics and experimental embryology. One instance of such evidence will, I think, eventually be found to be presented by the mode of origin of the lens in vertebrates.

MARCH 29, 1916

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PLATE 1

EXPLANATION OF FIGURES

3 to 5 Photomicrographs of transverse sections through the head of the embryo in figure 1. *b.*, brain; *l.*, lentoids; *m.*, mouth cavity; *L.*, lens; *p.*, plasma in oedematous pericardium; *r.l.*, retinal lentoid; *o.s.*, dissociated optic cup substance. × 150.

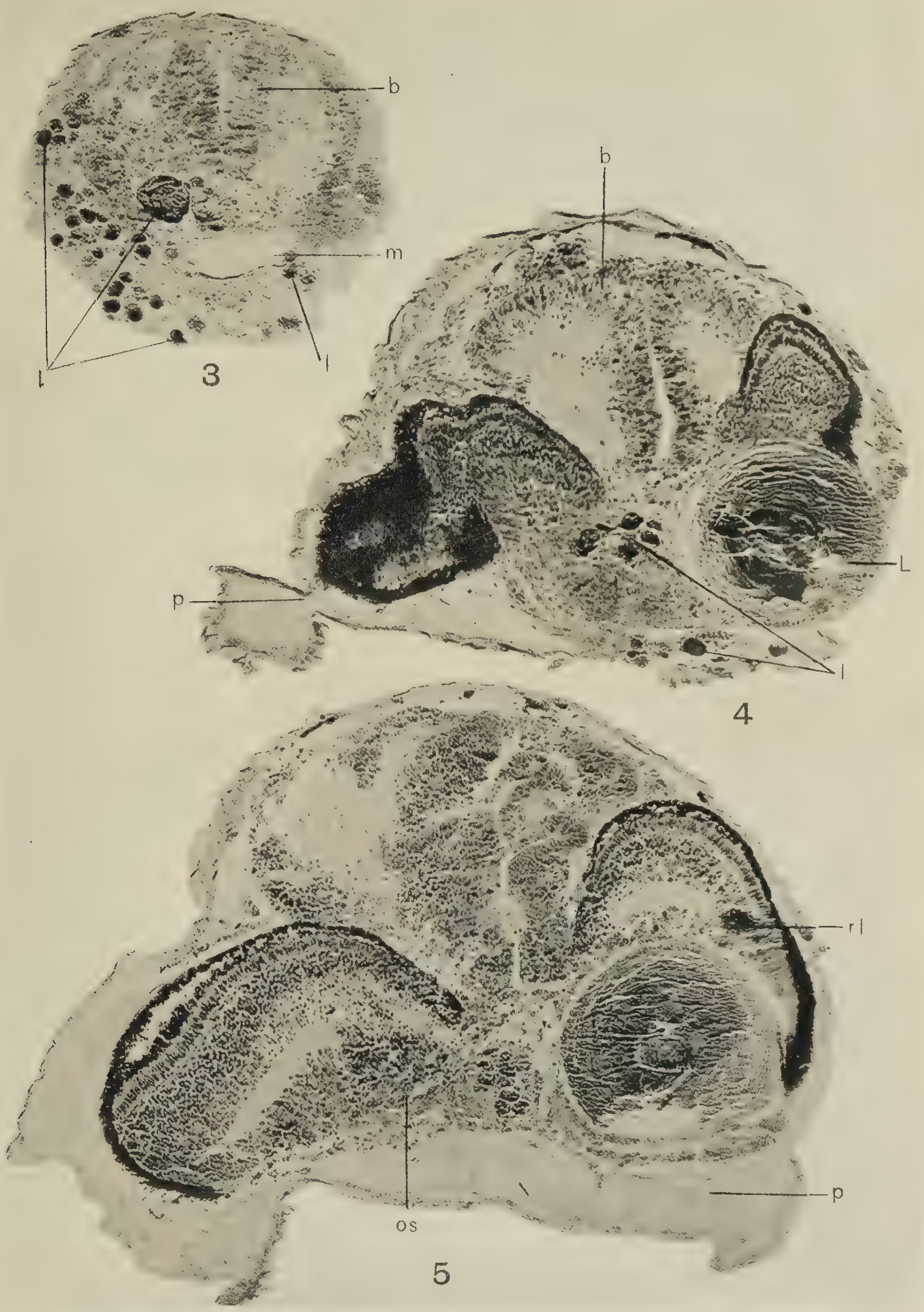
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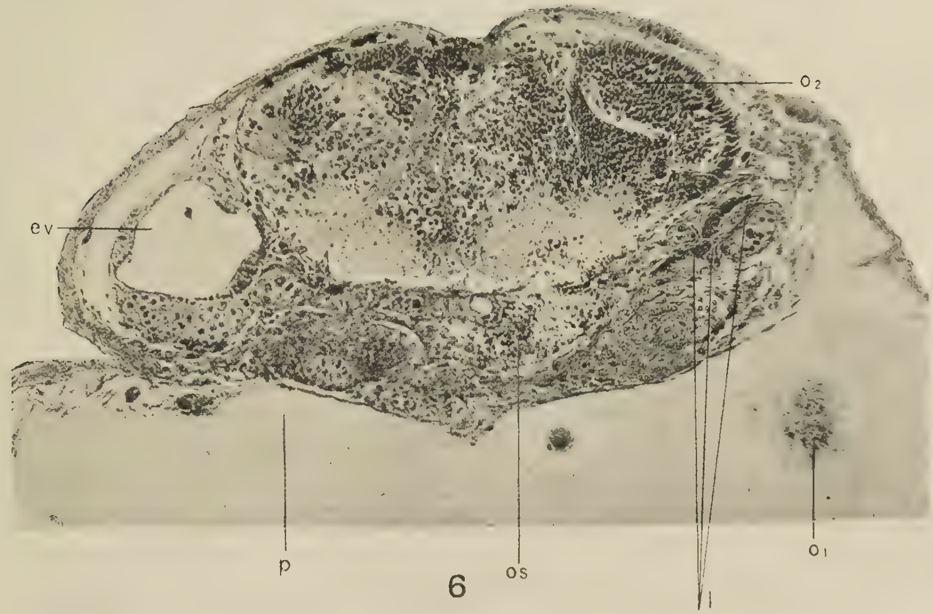
PLATE 2

EXPLANATION OF FIGURES

6 and 7 Photomicrographs of sections at more posterior levels of the same embryo. *o.s.*, dissociated optic cup substance in the oral cavity; o_1, the last trace of the right eye; o_2, the second optic cup of the right side; *l.*, lentoids; *p.*, plasma in pericardium; *e.v.*, ear-vesicle. × 150.



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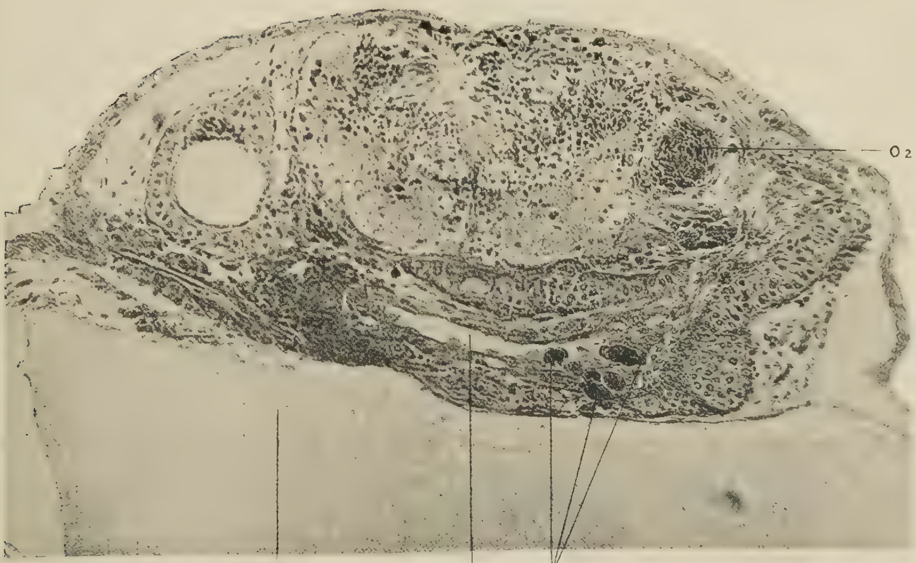

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A DEVICE FOR RECORDING THE PHYSIOLOGICAL RESPONSES OF SINGLE MELANOPHORES

R. A. SPAETH

From the Osborn Zoölogical Laboratory, Yale University

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In a previous paper[1] I have figured a curve showing the rhythmic pulsations of a single melanophore of *Fundulus* following a ten minute immersion in a 0.1 N solution of $BaCl_2$. This curve was obtained by measuring, with an ocular micrometer scale, the greatest diameter of a melanophore in the course of an hour, at intervals of from ten to thirty seconds. These empirical diameters were plotted against the time, giving a curve of pulsation. By using several microscopes with similar micrometer eye-pieces it was a simple matter to compare the effects of different stimulating solutions upon melanophores of the same fish. When the experiments lasted several hours and the movements of the pigment granules were relatively sluggish, this method proved more convenient than the automatic method described below. Frequently, however—as for example, in the case of a contraction by KCl or an induction current—the responses were so rapid that it became impossible to obtain an exact record of the pigment migration in terms of a series of diameters.

In order to obtain more accurate records of the physiological activities of the melanophores, the following graphic method was devised. The movements of the pigment granules in a single process of a melanophore were followed with an ocular micrometer carrying an adjustable scale. A cell was selected in which the processes were relatively long and straight. A single process was then brought to lie at right angles to the division of the micrometer scale with an empirically selected scale division just tangent to the outermost pigment granules (fig. 1a). When distal and proximal movements of the pigment granules occurred they were easily followed by turning the adjustment screw of the ocular micrometer. By means of a thin belt the turns of the adjusting screw were transmitted to a light set of pulleys and thence, by means of a cord winding about the pulley axis, to a heart lever and kymograph. A record of the pigment migration within a single process of the melanophore was thus obtained.

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[1] Spaeth: Journ. Exper. Zöol., 1916, xx, 193.

In every experiment a scale bearing the superficial layer of melanophores was immersed in the experimental solution contained in a diffusion chamber. The first type of chamber was made of glass slides. A 50 by 75 mm. slide formed the bottom; the sides were made of 25 by 75 mm. slides and the ends of cut slides of the same width, bound together by a modified Dehkotinsky cement which was found to have no appreciable effect upon the melanophores. A bent glass tube (not shown in the figure) to which a small rubber hose and clamp were attached, served as an outlet, while fresh fluid could be added either (1) directly, by a pipette, in cases where a measured quantity was desired, or (2) by means of a tube and funnel attached at a level of about 10 cm. above the bottom of the chamber. The position of a scale carrying the melanophores to be observed could be changed

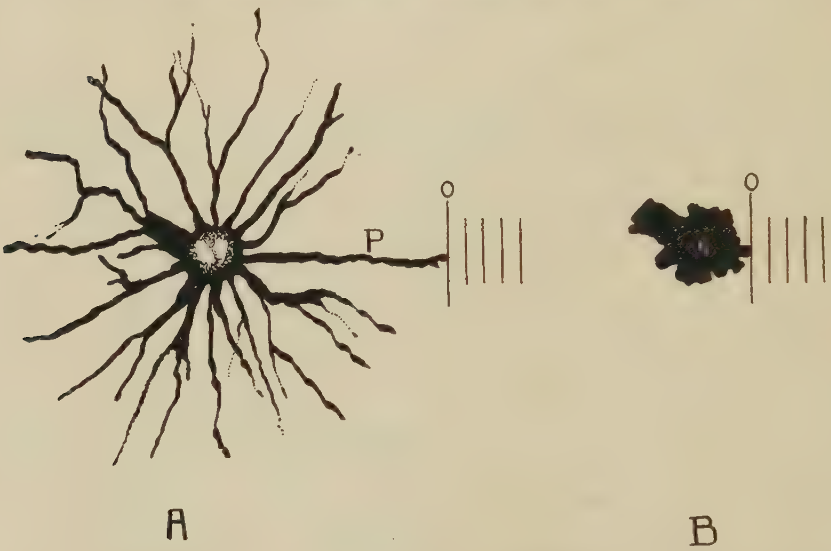

FIG. 1. Diagrammatic representation of the appearance of a cell just before stimulation, (*A*) and after the contraction is complete, (*B*). The division of the eye-piece micrometer marked *O* is kept tangent to the most distal pigment granules of the process *P* throughout the contraction by turning the adjusting screw of the ocular micrometer.

by means of a mechanical stage which carried the entire chamber. All observations were made with a 7 Leitz objective used as a water immersion lens.

In order to maintain a constant base line it was necessary to devise a mechanism for holding the melanophores immovably in position throughout the period of observation. This was accomplished as follows. A piece of fiber-composition was cut to span one end of the diffusion chamber, leaving a short flange projecting over each side. By means of a horizontal set screw (fig. 2 b) in one of these flanges, the bridge was firmly clamped across one end of the chamber. In the middle of the bridge a vertical set screw (fig. 2 c) raised or lowered the free end of a thin strip of spring brass (fig. 2 d) attached to the lower surface of the bridge. The free end of the brass strip carried a small two-pronged fork made of heavy platinum wires. Figure 2B shows how a scale is held in place against the bottom of the diffusion chamber by means of the flattened tips of the platinum wires. The platinum wires serve not only as a clamp for holding the scale with the melanophores in position but also as electrodes for faradic stimulation. Short pieces of copper wire were fused in a blast lamp to the proximal ends of the platinum wires and attached to two binding posts (*F*) on the fiber bridge. The two platinum wires were mutually insulated by means of a short glass rod lying between them. This rod was attached to them by cement and was similarly fastened to the free end of the spring brass. In order to avoid, as far as possible, the short circuiting of the secondary current through the salt solution between

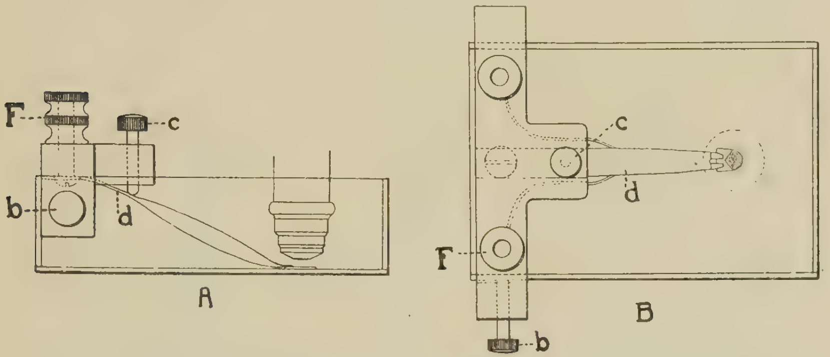

FIG. 2. Diffusion chamber and method of holding a scale in place during an observation. See text for details.

the prongs of the fork, the flattened ends of the platinum wires, with the exception of the lower surface of the tips, were coated with a thin layer of cement.

A second type of diffusion chamber was found to be very convenient in experiments demanding a rapid change of solutions. This consisted of a circular glass chamber with glass inlet and outlet tubes mounted on a heavy slide. The scale was kept in position in the middle of the circular chamber by means of a small piece of cover glass about 3 by 8 mm., one end of which rested upon the free, bony portion of the scale. By a touch of vaseline[2] the other end was made to adhere firmly to the bottom of the chamber. This arrangement was found particularly useful when stimulating with non-polarizable electrodes.

The movement of the ocular micrometer scale is normally accomplished by turning a milled head. This head was replaced by a small brass crank which enabled the operator to obtain a continuous and smooth motion of the micrometer scale. The rotation of the ocular micrometer screw shaft was transmitted to a set of wooden pulleys by a light belt. A piece of three-strand fish line with the ends spliced together gave very satisfactory results. The pulleys were so arranged that, in addition to the three transmission speeds, there were two winding spools for the cord carrying the heart lever. Various combinations of transmission speeds and winding pulleys gave magnifications on the kymograph drum ranging from 940 to 3850 diameters.

The device for carrying the writing lever was constructed as follows. A lead block (fig. 3, *L*) about 4.5 cm. long, carrying a heart lever, was arranged to run on parallel brass rods (*R*) the ends of which were screwed into a small iron base. A heavy piece of sheet brass (*S*) was soldered across the upper ends of the brass rods. By maintaining a constant distance between the rods, any possibility of sticking or of lost motion in the lead block was prevented. Through a hole in the middle of the brass piece (*S*) a cord (*C*) was passed and attached to an eye in the lead block. The other end of this cord was fastened to the winding pulley by a touch of sealing wax. By turning the adjusting screw of the ocular micrometer, the lead block carrying the writing lever was raised and lowered by the winding and unwinding of the cord attached to the pulley axis. The advantage of this arrangement as compared to an ordinary heart lever lies in the fact that since the writing tip moves in a plane perpendicular to the base line, every point of the contraction curve may be projected to the time-interval line, and, since it is unnecessary to apply corrections for a lever arc, the progress of the experiment may be read directly.

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[2] The vaseline has no injurious effect on the melanophores.

In experiments with salt solutions it was found that better results were obtained by momentarily raising the objective from the surface of the scale when the solution was being changed. The raising of the objective would obviously release the tension on the transmission belt, and the weight of the lead block would move the writing lever out of position. In order to maintain the position of the writing point, even when the objective was raised above the surface of the solution, a spring brake was attached to the pulleys, adjusted to overcome the rotary tension of the lead weight at the pulley axis.

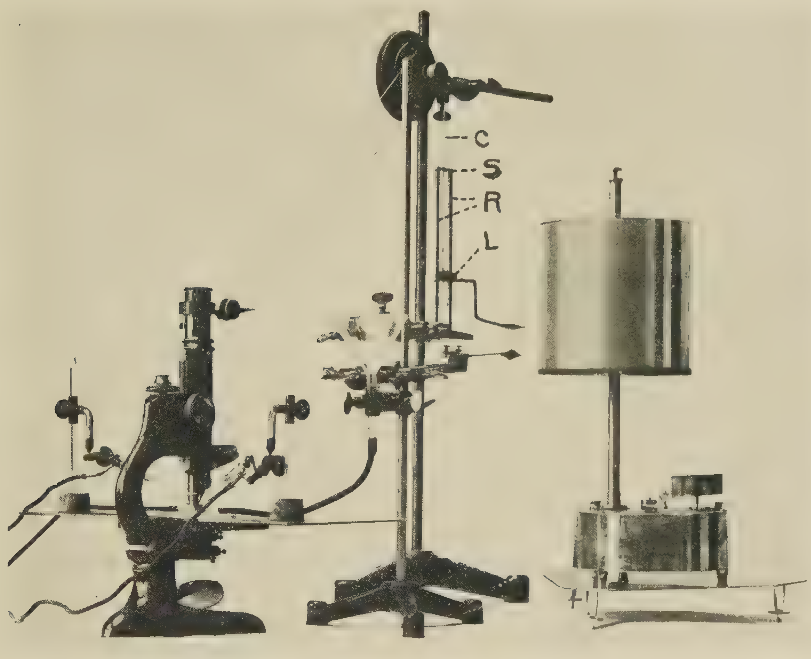

FIG. 3. Recording apparatus arranged for stimulation with non-polarizable electrodes. Letters explained in text.

At the beginning of an experiment the time and stimulation signals were aligned with the moveable writing lever as follows. The tip of the writing lever having been brought into contact with the lower edge of the drum, the eye-piece micrometer screw was turned until the writing lever had traced a single vertical line upon the smoked paper. The time and stimulus signals were now brought into contact with the kymograph paper with their tips on this vertical line. The angle of the vertical line with the base line was adjusted to 90° by means of a levelling table carrying the kymograph.

The most obvious disadvantage of this apparatus is that it requires constant attendance and manipulation by the operator, which, in addition to being time-consuming and laborious, introduces a personal equation into every observation. In most cases, however, the reactions are relatively so slow that, with a little experience, the pigment may readily be followed with the micrometer scale division. The advantage of the apparatus lies in that it affords an opportunity for recording graphically the physiological responses of single melanophores. Such graphic records and a discussion of their significance in the problem of muscle contraction will be found in another paper in this number of this journal.

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THE RESPONSES OF SINGLE MELANOPHORES TO ELECTRICAL STIMULATION

R. A. SPAETH

From the Osborn Zoölogical Laboratory, Yale University

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Preliminary

A considerable accumulation of evidence is at hand proving the melanophores of the lower vertebrates to be functionally modified smooth muscle cells. The responses of a variety of smooth muscle preparations to electrical stimuli exhibit certain characteristic peculiarities. If the melanophore is to be consigned to the category of smooth muscle, we should expect it to show the same peculiarities in its responses to electrical stimulation. In the absence of exact data concerning the reactions of single melanophores to electrical stimuli, and in the hope of confirming my previous conclusions, I have undertaken the experiments recorded in this paper. The results show that there is a close agreement between the responses of single melanophores and of typical smooth muscle preparations to faradic and galvanic stimulation. The evidence furnished by these experiments thus corroborates and amplifies the previous conclusion that the melanophore is to be considered a functionally modified type of smooth muscle cell.

PREVIOUS WORK UPON THE REACTIONS OF THE MELANOPHORES TO FARADIC STIMULATION

All the investigators (Buchholz (4), Pouchet (22), Lode (20), van Rynberk (29), Mayerhofer (21), von Frisch (30), Spaeth (26)) who have experimented with the effects of electrical stimulation in the skin of fishes agree that an induction current of sufficient duration and intensity produces a contraction of the melanophores. Such a contraction occurs whether the stimulus is applied directly to the surface of the fish or to an excised portion of the skin. Lode (l.c.) first observed the contraction of the melanin granules under the microscope in the excised fin of medium-sized trout.

The responses of amphibian melanophores to faradic stimulation have been less constant, though much of the conflicting evidence is to be ascribed to incorrect experimental premises. The earliest investigators (von Wittich (31), Harless (12), and Hering u. Hoyer (13), found that a tetanizing current produces a lightening of the skin of *Rana esculenta* which was reversible, the skin subsequently resuming its original dark color. Bimmerman (2), Êtérnod et Robert (8), Winkler (33), and Laurens (17) later made similar observations. Lister (19), Ehrmann (7) and others observed no effect from an induction current. The apparent discrepancy is not surprising since both Lister and Ehrmann considered the active phase of the melanophores to be the expansion. Their experiments were made upon light animals in which the melanophores were already contracted. With a strong tetanizing current the time required for the contraction of the melanophores in large larvae of *Amblystoma opacum* Laurens (l.c.) found to be at least fifteen minutes. Direct stimulation of small bits of excised skin gave similar results.

Faradic stimulation likewise produces a contraction in reptilian melanophores as was first shown by Bruecke (3) in the chameleon. The reaction in excised bits of skin is the same as in the intact animal. Both Bert (1) and Krukenberg (16) later verified Bruecke's observations. Keller (15), on the other hand, failed to observe any change in *Lacerta viridis* after stimulating with strong alternating currents. In the absence of any description of the experimental procedure it is impossible to judge the validity of this single negative instance.

From the foregoing briefly summarized experimental evidence, there seems to be no reasonable room for doubt that faradic stimulation produces a contraction of the melanophores of lower vertebrates. The exact nature of this contraction and its relation to other contraction phenomena has never been investigated.

EFFECTS OF FARADIC STIMULATION UPON SMOOTH MUSCLE

In the response to faradic stimulation both the latent period and the time required for a complete contraction may be said, in general, to be very much greater in smooth than in striated muscle. The effective liminal stimulus is also far greater for smooth muscle. This is strikingly illustrated by the classical experiment of Fick,[1] who found that an induced current of sufficient strength to produce a powerful tetanus in a frog's gastrocnemius or in the hand of the experimenter could pass through an adductor preparation of *Anodonta*, in the same circuit, without producing any sign of a contraction.

Wide variations occur both in latent period and contraction time in different types of smooth muscle. The following table, modified from Gruetzner, shows the range of such variations in a few examples of vertebrate smooth muscle. The figures have only an approximate value, however, since in each type of smooth muscle considerable additional variation occurs with a change in the strength and duration of the stimulus.

TYPE OF MUSCLE	LATENT PERIOD IN SECONDS	DURATION OF A SINGLE RESPONSE. (TIME OF CONTRACTION AND RELAXATION IN SECONDS)	OBSERVER
Stomach muscle of frog	1.5–10	60–120	Ranvier Schultz Gruetzner Winkler and others
Retractor penis of dog and horse	0.80–4.5	15–20 Contraction 90 Relaxation	Sertoli
Detrusor vesicae of cat	0.25	5–6 Contraction 35 Relaxation	Stewart
Membrana nictitans of cat	0.5	5–15	Lewandowsky
Ureter of rabbit	Fraction of second to 1 second	2–7	Engelmann

EXPERIMENTAL PROCEDURE

In studying the responses of the melanophores of *Fundulus* to faradic stimulation the procedure was as follows. After the fish[2] had been washed several times in tap water, distilled water and 0.1 N NaCl, or slightly modified Ringer solution,[3] a few scales were removed to 0.1 N NaCl or the modified Ringer solution, where they were left until the melanophores were widely expanded. A single scale was then transferred to a shallow depression slide or a diffusion chamber and was there held in place by means of a bit of cover slip and a touch of vaseline. The stimulus was applied through non-polarizable electrodes of the Zn-$ZnSO_4$ type. Drawn out tufts of absorbent cotton or camel's hair brushes served as contact points which were brought to lie on either side of the scale, with their tips 3 to 6 mm. apart. The most satisfactory results were obtained when the scale was covered by a piece of supported cover slip only slightly larger than the scale itself. The supports prevented mechanical stimulation by contact with the cover slip and the small size of the latter eliminated the possibility of an asphyxiation error due to an inadequate supply of oxygen. In many of the earlier experiments the scale was held in place and stimulated by means of a small, movable, two-pronged fork of flattened platinum wires. The effects of the polar products of electrolysis were largely eliminated by a strong current of NaCl or modified Ringer solution which maintained the C_{OH} far below a stimulating minimum.

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[1] Quoted from Gruetzner (11).

[2] Throughout these experiments 8.5 to 10 cm. females of *Fundulus heteroclitus* were used.

A series of preliminary trials showed that a record of the movements of the pigment granules in a single, unbranching process of a melanophore gave a fair picture of the contraction in the entire cell. All the graphs in the following pages represent the rate of movement of the most distal melanin granules in a single process of a single melanophore. Records of a series of responses in a single cell were always made by measuring the pigment migration in the same process.

RESPONSES OF THE MELANOPHORE TO FARADIC STIMULATION

Owing to the fact that the method of stimulation involves the passage of a considerable portion of the current through the mounting medium, it has proven impossible, up to the present time, to obtain a complete contraction of a melanophore as the result of a single make or break shock. It must, therefore, be borne in mind that the "single contractions" seen in the following curves are the result of a summation of make and break shocks.

A study of the responses of single melanophores to tetanizing currents of moderate intensity (fig. 1) brings out the following facts:

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[3] This modified Ringer was made up of 20 vols. 0.1 N NaCl + 1 vol. 0.1 N $CaCl_2$ + 1 vol. 0.1 N KCl.

1. The time required for a complete contraction and relaxation of the melanin granules in a process 0.1 mm. long is about of the order 90 to 120 seconds. This estimate is necessarily approximate since considerable variation occurs at the end of the relaxation phase.

2. The duration of the contraction phase is 25 to 30 seconds. Relaxation therefore requires from two to three times as long as contraction. The time relations for the contraction and relaxation in a single melanophore are thus approximately of the order seen in the case of a preparation of frog's stomach.

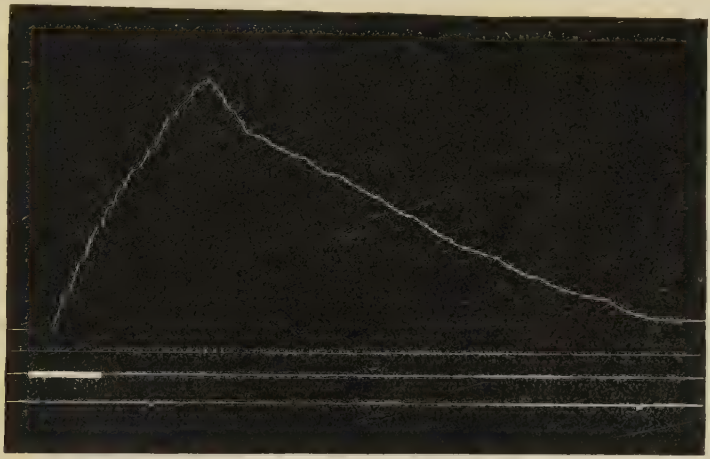

FIG. 1. Curve showing the response of a single melanophore to a brief tetanizing stimulus. The cell was removed to modified Ringer solution two hours and twenty minutes before stimulation began. The stimulus lasted nine seconds. After a latent period of 3 to 4 seconds the contraction began and lasted 21 seconds. The recovery was incomplete after 85 seconds. Upper line, time in seconds; middle line, stimulation period; lower line, time in minutes. *T* = ca. 22°C. The steps in the rising phase of the curve are due to the method of recording the pigment migration.

3. The steps and other irregularities which frequently appear in the curves are due to the peculiarities of the pigment migration. This migration is rarely a perfectly smooth flowing of melanin granules. The pigment frequently becomes momentarily blocked or jammed in the cell process forming a minute, opaque mass, from the proximal side of which single melanin granules, or groups and lines of granules, may be seen progressing toward the center of the cell. Such blocks

usually last from one to several seconds, though in extreme cases careful focussing will reveal several thin lines of pigment granules moving towards the center of the cell from one or more outlying blocks after the contraction is apparently completed. This phenomenon is especially likely to occur when the melanophores have been removed from the fish for several hours.

THE ORIGIN OF THE CONTRACTION WAVE AND THE LATENT PERIOD

The study of a large number of contraction curves has shown that in the response to a tetanizing current of moderate strength at room temperature there appears a characteristic latent period of 4 to 6 seconds. Other observers have recorded somewhat longer latent periods in several species of fish. Thus Lode (l.c.) found in the case of the trout that the contraction followed ½ to 1 minute after stimulating, whereas Mayerhofer (l.c.) noted a somewhat briefer latent period for *Esox*, 20 to 30 seconds, depending upon the strength of the current. In *Crenilabrus pavo* von Frisch (l.c.) found a distinct reaction in the melanophores 5 seconds after stimulating. It is significant that the latent period is in every case a long one, such as we might expect to find in a relatively sluggish tissue, like the smooth muscle of the frog's stomach.

By the latent period is meant the time that elapses between the application of the stimulus and the first sign of a contraction of pigment granules at the distal end of the process under observation. The above time relations apply only in the case of the distal pigment granules. The latent period for melanin granules lying near the proximal ends of the cell processes is less than half as great as for the distal granules. In other words the contraction wave begins at the center of the cell and spreads peripherally. The consistent difference in latent period at distal and proximal ends of the same processes in one melanophore is shown in the table below. The times were recorded on two stop watches, and different processes were selected in order to give the observations a random value.

Ex. Sp. 16–41. T. = 20°C. March 31, 1916.

9.00 a.m. Scales from a 10 cm. female to modified Ringer.

9.40 a.m. Stimulation began; 3 storage cells in series; secondary of Harvard inductorium at 6 cms., non-polarizable electrodes. Each stimulus lasted 6 to 10 seconds. Between stimuli the cell was permitted to reexpand fully. Contractions began after the following time intervals in seconds:

	Proximal		*Distal*
	2.0		5.0
	1.8		4.4
	1.8		4.4
	2.0		6.0
	2.2		4.4
	2.0		5.2
	1.6		6.2
	2.2		7.8
	1.6		4.6
	2.2		5.6
Average	2.0	Average	5.4

The average distance between the proximal and distal points in the processes at which the migration first appeared was about 0.075 mm. The difference between the average times for the beginning of the contraction at the center and the periphery of the cell—i.e., $5.4-2.0=3.4$—gives a value for the time required for the contraction wave to traverse a distance of 0.075 mm. A simple calculation shows that the speed of propagation of the contraction wave within the melanophore, under the above experimental conditions, is therefore only 22 μ per second, or 7.9 cm. per hour. This extremely slow rate seems to leave little room for doubt that we are here dealing with a passage of the stimulus through the substance of the melanophore and not along a broken branch of sympathetic nerve fiber. The origin of the contraction wave at the center of the cell suggests the possibility of the transmission of the tetanizing stimulus to the cell through a broken nerve fiber. The stimulus having reached the center of the cell, however, its further propagation appears to be independent of finer nerve fibrillae.

In the response to a tetanizing current of moderate strength, the peak of the contraction curve appears distinctly pointed, never rounded as in typical contraction curves of smooth and of striated muscle. An examination of the behavior of the melanin granules in a single process of the stimulated melanophore shows that near the end of the contraction phase a relaxation wave frequently originates at the center of the melanophore before the most distal of the pigment granules have completed their centripedal migration. At the proximal end of a process there thus appears a slowly travelling, distally moving wave of relaxation, which gradually encroaches upon and carries with it the proximally moving melanin granules. The sharp peak of the recorded contraction marks the point at which the relaxation wave has enveloped the last of the contracting pigment granules. In the

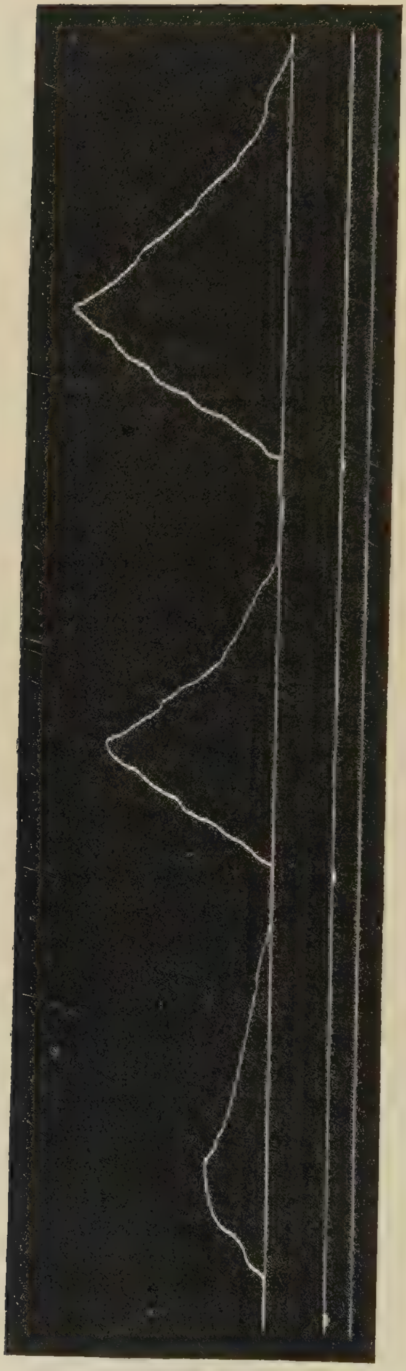

FIG. 2. Shortening of the latent period and increase in the height of the contraction following a decrease in the distance of the secondary coil from the primary. First curve 33 minutes after removal from the fish with secondary at 7 cm.; latent period, 8 to 9 seconds. Second curve 35 minutes after isolation from the fish, secondary at 5 cm.; latent period 3 to 4 seconds. Third curve 39 minutes after removal from the fish, secondary at 3 cm.; latent period 2 to 3 seconds. Time in seconds; $T = 24°C$.

melanophore we thus find that the relaxation phase may be initiated before the completion of the contraction. Thus it appears that both contraction and relaxation originate at the center of the cell, though why this should be so is not at present evident. There may exist a correlation of some sort between this central origin of contraction and relaxation waves and the centripetal direction invariably taken upon stimulation by the pigment granules.

By comparing in a number of curves the time required for a complete contraction and the actual distance traversed by the migrating pigment granules, it appears that the speed of the moving melanin granules is about 3 μ per second or 1.08 cm. per hour. This is approximately one-eighth as fast as the propagation of the contraction wave (7.9 cm. per hour).

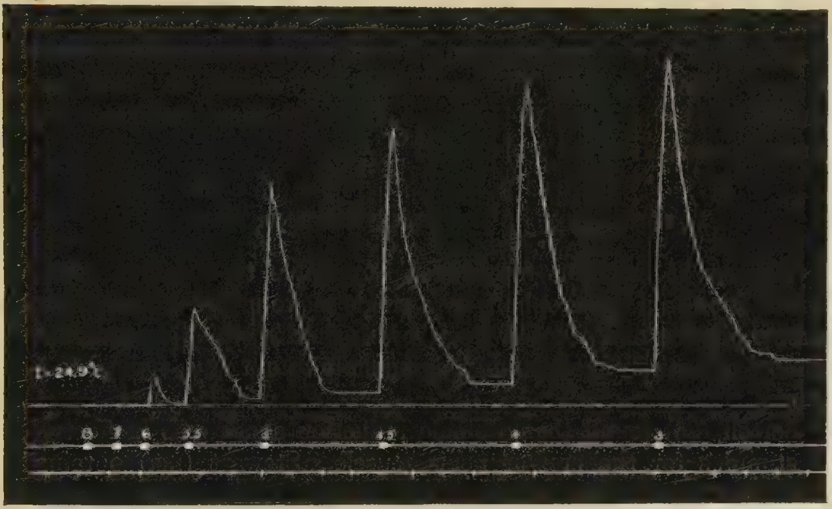

FIG. 3. Curve of increasing response with an increase in the strength of stimulus. The figures represent the distance in centimeters of secondary from primary. Stimulation began two hours and nine minutes after removing the cell from the fish. Time in minutes.

THE EFFECT OF INCREASING THE STRENGTH OF THE STIMULUS

Figures 2 and 3 show the effect of increasing the strength of the stimulating current by shortening the distance of the secondary from the primary coil. In figure 2 are shown three curves representing consecutive contractions in the same cell with the secondary at 7, 5 and 3 cm., respectively, from the primary. The stimulus lasted two seconds. The latent period becomes progressively shorter, falling from 8 to 9 seconds to 3 to 4 seconds and finally to 2 to 3 seconds. At the same time the speed and extent of the pigment contraction increases to the maximum shown in the third curve. Similar conditions obtain when a weaker stimulus is used.

Figure 3 shows the successive responses of the same cell to tetanizing currents of gradually increasing intensity. Owing to the slow rate of the drum the differences in the latent period are obscured, but the increased height of the contraction with the increase of the stimulus is very definite. These curves are strikingly similar to the series obtained by Stewart (p. 192, fig. 6, loc. cit.) upon increasing the stimulus in a preparation of cat's bladder.

SUMMATION OF CONTRACTIONS AND TETANUS

Whereas it has thus far proven impossible to obtain a complete contraction in a melanophore by a single make or break shock, by the proper spacing of a series of single break shocks[4] which are individually almost or entirely ineffective, it is possible to obtain a typical tetanus curve.

Sertoli (25) found that a single induction shock was ineffective as a stimulus for the erector penis muscle. Stewart (l.c.) obtained a complete fusion of the responses to moderate break induction currents in the cat's bladder when the interval between the successive stimuli was reduced to one second.

The summation record for the melanophore (fig. 4) shows that with a shortening of the interval between stimuli, summation appears and becomes increasingly pronounced until, with an interval of 2 to 3 seconds, complete fusion occurs. Except for the time relations, this curve is precisely like the tetanus curve obtained from smooth and striated muscle preparations.

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[4] All the experiments in this series were carried out with the help of a modified Martin key. At the suggestion of Professor E. G. Martin the current was made and broken by means of a platinum needle dipping in a vial of clean mercury covered with alcohol. The contact spark was reduced to a minimum by inserting a small telephone condenser of 1 microfarad capacity across the primary terminals.

No refractory period has been observed in the melanophore, a stimulus being effective in every phase, both of the contraction and relaxation. Stewart (l.c.) has observed a similar absence of a refractory period in preparations of cat's bladder.

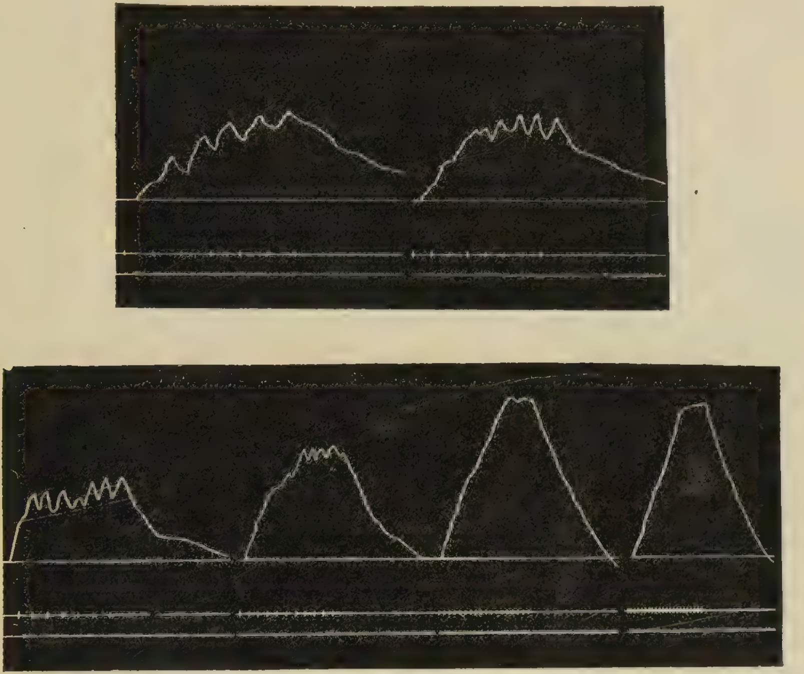

FIG. 4. Development of tetanus in a single melanophore. Powerful break induction shocks were applied at intervals of 15, 10, 8, 5, 3, and 2 seconds respectively. Stimulation began one hour and twenty-seven minutes after the removal of the cell from the fish. Time in seconds.

PREVIOUS WORK ON THE EFFECTS OF A CONSTANT CURRENT UPON THE MELANOPHORES

Experiments with the effects of a constant current upon melanophores are few in number and unsatisfactory in results. Lister (19) found that the melanophores of *Rana temporaria* failed to respond to galvanic stimulation. He believed that the absence of response in his experiments was only apparent and that, in reality, the chromatophores were paralyzed in the condition they happened to be in at the time of stimulation, thus eliminating the possibility either of a contraction or of an expansion. Hermann (14) could detect no color changes in frog larvae following galvanic stimulation. More recently Winkler (33) studied the effect of a galvanic current upon the melanophores of *Hyla.* A small piece of skin was transferred from the back of the animal to physiological salt solution and was then subjected to galvanic stimulation under the microscope. Under these circumstances Winkler observed an expansion of the melanophores —"schwarze Faeden" appearing from the deeper portions of the preparation. As to the reversibility of this process he states (p. 257):

> Beim Aufhoeren des galvanischen Reizes verschwinden die Faeden; es ist aber dabei nicht zu entscheiden, ob dieses Verschwinden durch ein Zurueckziehen der Fortsaetze oder durch ein Fortwandern des pigments erfolge.

Presumably the finer details of a pigment migration were not visible in Winkler's preparation.

The relaxation of amphibian melanophores in isolated bits of skin observed both by Winkler (l.c.) and Laurens (17) following galvanic stimulation is probably to be interpreted as the effect of OH ions liberated at the cathode. Laurens (l.c.) has recorded an expansion both in whole larvae and in small, excised pieces of skin of *Amblystoma opacum* when stimulated by a constant current through platinum electrodes.

He says (p. 611):

> When bits of isolated skin with contracted melanophores are thus stimulated on a glass slide with a current of from 3 to 4 m.a., and for about five minutes, the melanophores all expand. All attempts to demonstrate a greater effect of one pole in these expansion effects resulted negatively. . . . In two cases out of ten experiments with bits of isolated skin it was thought that the expansion at the cathode began earlier and was more extensive than at the anode, but this is not sufficient evidence for ascribing to that pole a greater effect.

From previous experiments with a variety of physiological stimuli (Spaeth, 26) I have been led to believe that the contracted phase of the melanophore is that of stimulation. It seemed strange, therefore, that a constant current should produce a *relaxation* in amphibian melanophores. Believing the polarizable electrodes of Laurens and Winkler[5] to be a possible source of error in their experiments, I first made a number of preliminary trials with *Fundulus* melanophores using a constant current delivered through platinum electrodes. Scales immersed in 0.1 N KCl were subjected to galvanic stimulation from one, two and three 2-volt storage cells. The first experiment showed that, under these experimental conditions, a relaxation of the melanophores begins immediately. In subsequent experiments a drop of neutral red added to the KCl solution showed, furthermore, that the expansion invariably begins at the cathode. Under the low power of the microscope it was a simple matter to observe the color change of the neutral red at the cathode. The yellow area of alkalescence gradually diffused toward the anode and a short distance behind the periphery of the yellow semicircle the melanophores could be seen to be expanding. By the time the alkaline region had extended to the anode all the melanophores were either fully expanded or showed a distinct beginning of an expansion. When a scale was transferred from the now alkaline KCl solution to neutral KCl all the melanophores on the side of the scale towards the anode contracted completely. Thus in the region where the exposure to OH ions had been shortest and where the hydroxyl concentration was at a minimum the relaxation was reversible. All the melanophores in the region of the cathode, however, remained expanded and soon disintegrated (fig. 5). Fifteen minutes after having been returned to neutral KCl the scale appeared half dark and half light.

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[5] Presumably Winkler used polarizable electrodes since he makes no statement to the contrary.

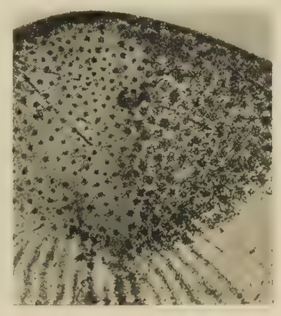

FIG. 5. Median portion of a scale showing the effect of stimulating with a constant current through platinum electrodes placed on either side of the scale. The melanophores towards the cathode are expanded by the action of the liberated OH ions.

From this first set of preliminary experiments it seemed that when the melanophores are stimulated with a galvanic current, using platinum electrodes, the relaxation that appears is due, *not* to the electrical stimulus, but to the effect of OH ions liberated at the cathode. The following experiments supported this conclusion. Two depression slides were placed side by side, filled with $\frac{1}{2}$ cc. 0.1 N KCl and connected by a narrow bridge of filter paper, supported by a cover glass set on edge between the slides. A scale with contracted melanophores was now placed in each chamber. After adding a small drop of aqueous neutral red solution to the KCl solution in each chamber, a platinum electrode was brought into contact with the bony portion of each scale. A constant current (three storage cells) was now passed for five minutes from one scale over the moist filter paper bridge to the other. The diffusion from the cathode to the anode was largely prevented by the filter paper bridge. In about two minutes all the melanophores of the scale in the cathode chamber were fully relaxed, while those in the anode chamber remained contracted. The neutral red solution had meanwhile turned yellow at the cathode and was becoming colorless at the anode on account of the bleaching effect of the liberated Cl_2 or O_2. At the close of the experiment, the scales and the electrodes were removed and, without disturbing the solutions in the two depression slides, a fresh scale with contracted melanophores was brought into each chamber. Fifteen minutes later, no current having passed meanwhile between the chambers, all the melanophores of the scale in what had previously been the cathode chamber were completely expanded while those of the scale in the anode chamber remained contracted. The two scales were thereupon fixed in absolute alcohol. They are shown in figures 6 and 7.

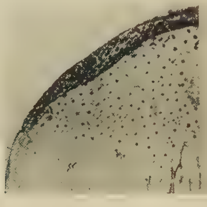

Fig. 6

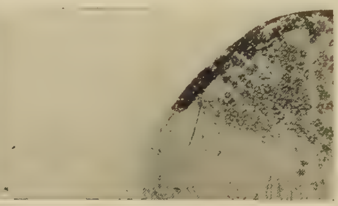

Fig. 7

Fig. 6. Anodal products of electrolysis of KCl solution give no relaxation. See text for details.

Fig. 7. Hydroxyl ions produced at the cathode cause relaxation of melanophores in KCl. See text for details.

From these two experiments it is evident that the expansion of melanophores which is initiated at the cathode upon stimulation with a galvanic current and polarizable, platinum electrodes is effected by the OH ions and not by the passage of the constant current through the cells. Finally, experiments were carried out with solutions of KOH in 0.1 N KCl which showed that, beyond a definite OH concentration, a relaxation of the melanophores invariably occurs.

THE EFFECT OF THE CONSTANT CURRENT ON SMOOTH MUSCLE

Practically all experimenters[6] on smooth muscle preparations agree that both the make and the break of the constant current act as stimuli and produce contractions. The make contraction is larger than the break. Stewart (l.c.) has shown conclusively that in the cat's bladder the *flow* of the current also acts as a contracting stimulus. In his experiments the current (about 3 m.a.) was made, allowed to flow for a few seconds, and then broken. The result was a strong make contraction succeeded by a period of very slow, imperfect relaxation, the true relaxation occurring only after the break contraction. That the tonic elevation was actually due to the flow of the current was shown by making the current in the same way but shutting it off by means of a rheonome. In this way the influence both of flow and of break was eliminated. The curve showed a strong make contraction followed by a complete relaxation.

In spite of Stewart's proof to the contrary the impression continues to exist that smooth and striated muscle are alike in their response to the constant current. Thus du Bois Reymond (5) states (p. 554):

> Bei der Reizung mit dem konstanten Strom gilt zunaechst wie bein gestreiften Muskel, dass nur Schliessung und Oeffnung, jede als ein einzelner Reiz, wirksam sind, waehrend die Durchstroemung an sich keinen Reiz bildet.

In the case of the melanophores of *Fundulus* no response has as yet been observed to the breaking of the constant current, but both make and flow cause a contraction, as was the case in Stewart's experiments on the cat's bladder.

I have recently carried out a series of experiments upon the melanophores of *Fundulus* using non-polarizable electrodes of the Zn-$ZnSO_4$ type. Stimulation was effected by means of fine camel's hair brushes set in plaster caps covering the ends of the heavy glass tubes containing the concentrated solution of zinc sulphate. The plaster caps were made up fresh for every experiment or short series and in no case were the electrodes used longer than two hours at a time. The plaster was mixed with 0.1 N NaCl solution and, after setting, both electrode caps were saturated with 0.1 N NaCl. As an additional precaution the NaCl from the diffusion chamber, in which an experiment had been carried out, was tested for SO_4. The non-polarizable electrodes were most conveniently manipulated by having their adjustable uprights mounted on a sheet of glass (figure 3 of the previous paper in this number of this JOURNAL.) A depression slide or shallow diffusion slide containing about 0.5 cc. 0.1 N NaCl and a scale with expanded melanophores was brought below the objective on the sheet of glass on the stage of the microscope. The electrodes were then swung into position and the final adjustment of the single melanophore to be studied was accomplished by moving the sheet of glass carrying both the electrodes and the diffusion chamber. The direct current of the city circuit was reduced by means of a long tube containing one movable and one fixed copper electrode and a dilute solution of copper sulphate. The strength of the current was varied by sliding the movable electrode back and forth in the tube. The actual current that stimulated the single cell could not be accurately measured since the electrodes simply dipped into the solution containing the melanophores.

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[6] Gruetzner (l. c.) gives an extended review of the older papers on this subject.

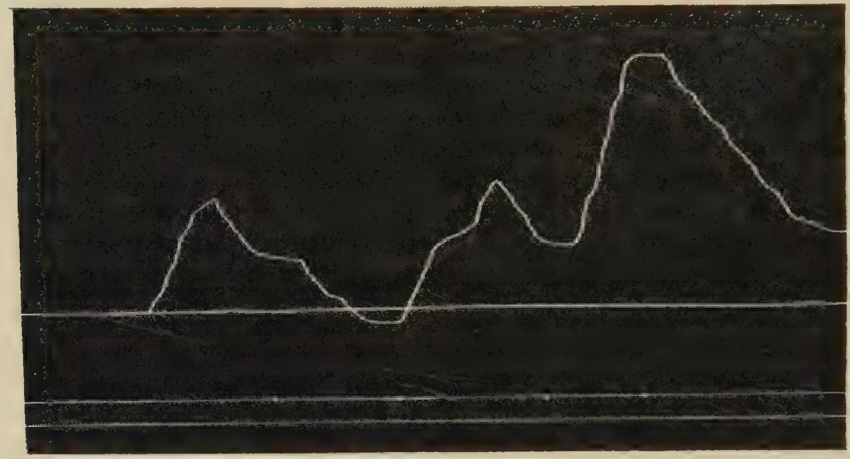

FIG. 8. Effect of a weak constant current through non-polarizable electrodes. Stimulation began 45 minutes after removing the cell from the fish to 0.1 N NaCl. Time in seconds. $T = 21.3°C$. Detailed explanation in text.

The responses of the melanophores to a constant current of medium strength show a striking correspondence to the reactions of smooth muscle preparations under similar experimental conditions (fig. 8).

There is a sharp rise at the make after a very brief latent period

(<2 seconds) followed by a gradual and partial falling off of the contraction, giving a characteristic plateau. Stewart (fig. 9, p. 196, l.c.) recorded precisely this type of curve for the response of the cat's bladder. He noted further a distinct response in the bladder preparation when the constant current was broken, which I have never observed in the melanophore. There is, however, a latent period of about 10 seconds before the relaxation begins which may represent a suppressed influence of the break shock.

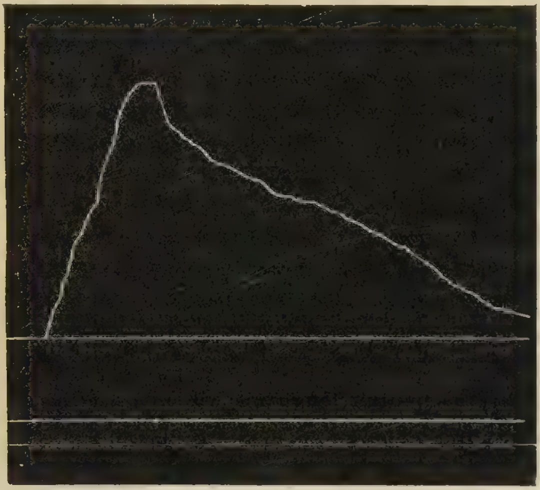

FIG. 9. Curve showing the response of a melanophore to a strong constant current through non-polarizable electrodes. A complete contraction occurs which continues during the *flow* of the current.

The second part of figure 8 represents the effect of the flow of the constant current. A stimulus of medium strength was sent into the preparation and, after the plateau had appeared (at the fourth signal), the current was gradually increased by means of the sliding electrode of the copper sulphate rheonome. In this case there was no additional make stimulus, yet the melanophore contracted completely and remained contracted during the flow of the stronger current. At the fifth signal the current was broken and a gradual expansion of the pigment followed.

When the stimulus is sufficiently strong the melanophore contracts completely and remains contracted until the breaking of the current.

This condition is illustrated in figure 9, which shows the effect of a strong constant current. The latent period is again very short (<2 seconds) and the contraction persists during the flow of the current, as is shown by the flat top of the curve. In this case the contraction represents the response to a combination of make and flow stimuli.

DISCUSSION OF RESULTS

The striking correspondence between the curves recorded here and the contraction curves of a variety of smooth muscles would not in itself be significant. The pseudopodia of an amoeba or a heliozoan might exhibit contraction phenomena of the same general order. However when this correspondence is considered in conjunction with other morphological and physiological characters of the melanophore such, for example, as the sympathetic innervation, the sensitivity to adrenalin and certain other drugs, the power of rhythmic pulsation, the ability to maintain a condition of increased tonus for protracted periods,[7] etc., we are justified in attaching greater significance to the similarity.

In view of a considerable amount of verbal criticism regarding the earlier contention that the melanophore is to be considered a functionally modified smooth muscle cell it may be well to include here a brief statement of the author's position.

We apply the term "smooth muscle" to a variety of tissues which correspond to a number of empirically selected morphological and physiological characteristics. Investigation has shown that the melanophore conforms to *all* the criteria by which we recognize smooth muscle. Since the empirical criteria that establish the category of smooth muscle and the universal characteristics of smooth muscle are identical, it follows that the melanophore exhibits all the characteristics of smooth muscle. Hence it seems logical to speak of the melanophore as a type of smooth muscle cell. The insistence upon the nomenclature is significant in so far as further study of the melanophore may throw light upon certain obscure phases of the problem of muscle contraction.

SUMMARY OF RESULTS

1. The melanophores of *Fundulus heteroclitus* fail to contract completely when stimulated by a single make or break induction shock.

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[7] The melanophores in a living fish will apparently remain light over a light bottom indefinitely. I have kept *Fundulus* in a white aquarium for several months and have observed no tendency to turn dark during this period.

2. A tetanizing current of moderate intensity produces a complete contraction in which the rising phase lasts 25 to 30 seconds and the relaxation 65 to 90 seconds. Contraction and relaxation are completed in 90 to 120 seconds.

3. The form of the contraction curve is never smooth as in a curve of typical muscular contraction for the pigment granules exhibit a certain amount of irregularity and blocking in their migration within the cell processes.

4. The contraction wave originates at the center of the cell and passes to the periphery at the rate of about 22 μ per second.

5. The most rapid pigment migration thus far observed in response to electrical stimulation is 3 μ per second.

6. With a moderate tetanizing current there appears a latent period of about 5 seconds at the distal ends of the cell processes whereas nearer the proximal ends of the processes the latent period is only about 2 seconds.

7. With an increase in the strength of the stimulus there appears a progressive shortening of the latent period and an increase up to a maximum in the height of the contraction.

8. By the proper spacing of single break shocks which are individually almost or entirely ineffective as contracting stimuli, there appears a summation of stimuli. Eventually, with an interval of 2 to 3 seconds, complete fusion occurs, giving a typical tetanus curve.

9. The relaxation of the melanophores observed by previous investigators upon stimulation with a constant current and polarizable electrodes is probably brought about by the hydroxyl ions liberated at the cathode and is not the result of galvanic stimulation.

10. By using non-polarizable electrodes it appears that both the make and the flow of the constant current act as contracting stimuli. No response to the breaking of the constant current has thus far been observed.

11. In its responses to electrical stimulation the melanophore shows all the characteristics of smooth muscle. The experiments recorded in this paper thus corroborate and amplify the author's former conclusion that the melanophore is to be considered a functionally modified type of smooth muscle cell.

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EXPERIMENTAL STUDIES ON THE ORIGIN OF MONSTERS[1]

I. AN ETIOLOGY AND AN ANALYSIS OF THE MORPHOGENESIS OF MONSTERS[2]

E. I. WERBER

Osborn Zoological Laboratory, Yale University
(From the Zoological Laboratories of Princeton and Yale Universities)

EIGHTY-NINE FIGURES

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[2] Brief reports of the results of this work have been presented and demonstrations of material made at the meeting of the American Society of Zoologists in Philadelphia (December 29 and 30, 1914) and at the meeting of the American Association of Anatomists at New Haven (December 28–30, 1915). A preliminary communication was also published in the Anatomical Record (cf. Werber '15 c.)

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I. INTRODUCTION

A review of the literature shows us that we are at the present time confronted with two theories regarding the causal genesis of monsters. The first and older one, the amniotic theory, is essentially a mechanical theory, while a more recent one which is based on recent experiments on the influence of chemical alterations of the egg's environment, might be spoken of as the chemical theory of teratogenesis.

The amniotic theory maintains that terata are due to anomalies of the amnion, which latter, by adhering too closely to the embryo or constricting it, is thought to bring about the various well known malformations. The inadequacy of this assumption has in recent years been repeatedly pointed out.[3] It is a well known fact that amniotic anomalies are found relatively rarely in malformed ova. In one hundred and sixty-nine pathological ova which Mall ('08) has examined he asserts that he has found not a single case of anomaly of the amnion. Wherever amniotic adhesions are found, Mall, I believe rightly regards them as incidental. It is not impossible that they may be syngenetic with some terata, i.e., due to the same causes which brought about the developmental deviation of the embryo. This might particularly apply to the experimental terata of Dareste ('91) and others, in which anomalies of the amnion were found. On the whole, it might well be said, that in view, particularly, of experimental results on anamniotes, the amniotic theory would hardly seem to deserve more than historical interest.

In rejecting this mechanical theory Mall traces monstrous development to faulty implantation of the ovum in a diseased uterus, which in turn makes adequate nutrition of the embryo impossible. This is, essentially, a chemical theory of teratogenesis for it assumes the atypical development of the embryo to be due to lack of necessary substances.

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[3] Cf. Mall ('08) and Jordan ('09).

More direct support for the chemical theory of teratogeny is offered by the recent investigations of Stockard ('07, '09, '10 a) and McClendon ('12 a and b), both of whom obtained one-eyed monsters by subjecting developing teleost eggs to the action of various toxic substances, such as magnesium chloride, alcohol, ether, alkaloids, etc. These investigations have shown that some such developmental deviations as are found to occur spontaneously may be brought about by the chemical action of various substances, and thus they suggest that atypical development in nature may be due to pathochemical alterations of the germ's environment.

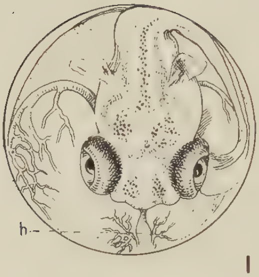
The above considerations have led me to assume that in order to attack the problem of atypical development in nature effectively it is necessary to find the unusual chemical factors which cause the embryo in its natural environment to develop in a defective or monstrous manner.

Since the metabolism is the greatest source of chemical modifications of the body, I concluded that the solution of the problem of the causal genesis of monsters must be sought for in pathologic parental metabolism.

Starting from this assumption, I carried out, in the summer of 1914, some experiments on Fundulus heteroclitus, the fertilized eggs of which were subjected to the action of solutions of urea, butyric acid, lactic acid, acetone, sodium glycocholate and ammonium hydroxide. Conclusive results were so far obtained only with butyric acid and acetone. The (rather simple) methods employed have been described in a former paper (Werber '15 c) to which the reader is referred.

II. THE RECORDED TERATA

The results which were obtained are very much alike in both series of experiments, with butryic acid and acetone. The variety of deformities being almost endless in both, it would be practically impossible to present much more than certain types



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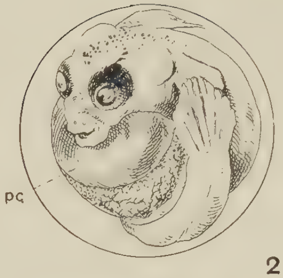

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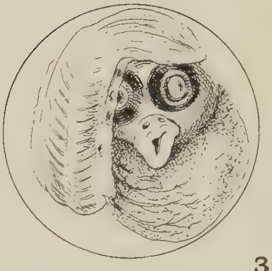

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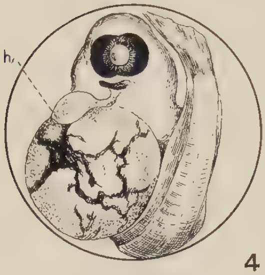

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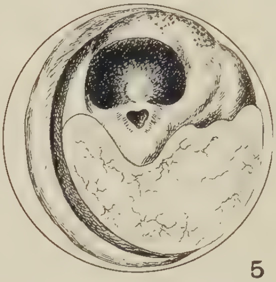

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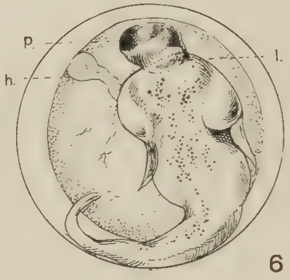

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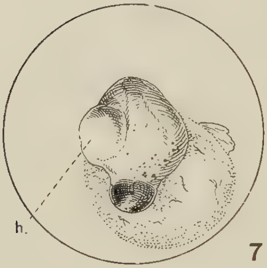

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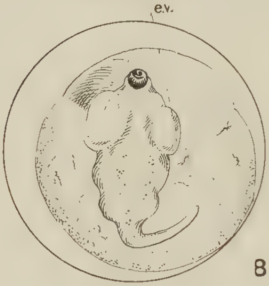

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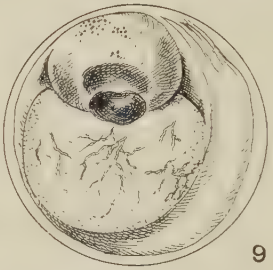

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Fig. 1 Normal embryo of Fundulus heteroclitus, nine days old. *h.*, heart.

Fig. 2 Synophthalmia bilentica, from $\frac{1}{12}$ gram molecular butryric acid, twenty-eight days old. *pc.*, pericardial vesicle.

Fig. 3 Snyophthalmia bilentica, from $\frac{1}{12}$ gram molecular butyric acid solution, twenty-eight days old.

Fig. 4 Synophthalmia unilentica, from $\frac{1}{12}$ gram molec. butyric acid, twenty-four days old.

Fig. 5 Cyclopia synophthalmica, from acetone solution (35 cc. to 50 cc. sea-water) twenty-six days old.

Fig. 6 Cyclopia perfecta, from $\frac{1}{12}$ gram molec. butyric acid, twenty-four days old. *l.*, lens; *p.*, pigment epithelium; *h.*, heart.

Fig. 7 Cyclopia perfecta, from acetone solution (35 cc. gram molec. to 50 cc. sea-water) twenty-five days old.

Fig. 8 Cyclopia perfecta, from $\frac{1}{16}$ gram molec. butyric acid, thirteen days old. *e.v.*, ear vesicles.

Fig. 9 Cyclopia perfecta, from $\frac{1}{12}$ gram molec. butyric acid, twenty-eight days old.

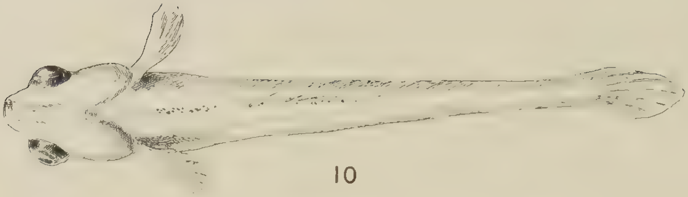
of them. In a previous publication (Werber '15 c), I have presented a brief survey of the recorded terata. While a more complete presentation of them is attempted in this paper, I am fully convinced that it is inadequate to convey the proper impression of the great range of variation in the noted effects.

1. *Terata of the head*

The malformations of this part of the body are many and unusually varied. Indeed, as will be pointed out later, this part of the body appears to be the most susceptible one to the influence of toxic solutions. The deformities of the head affect the sense organs, the brain, the mouth, and the skull. All of them are usually found to occur in various combinations. The most striking ones on examination in toto are those that concern the eyes.

a. The ophthalmic terata. When Fundulus eggs are subjected to the action of butyric acid or acetone in the concentrations stated above, embryos with normal eyes are of the rarest occurrence. Cyclopia, i.e. the presence of a single median eye is found very often. I have likewise recorded in my observations a wide range of intermediate stages between two normal eyes in the typical position in the head all the way down through more or less closely approximated eyes or eyes of an apparent double composition and true cyclopia to complete anophthalmia as described so often by the older teratologists and as obtained experimentally in recent years by Spemann ('04), Lewis ('09), Stockard ('09, '10 b), and others.

A comparison of figures with the normal embryo in figure 1 is very instructive of this gradual transition from the normal eyes to various synophthalmic or cyclopean defects. In figure 2 an embryo is seen with eyes apparently normal, but for their position. They are located on the frontal part of the head in approximation to one another and, while not being fused externally, they are found to be so on examination of sections at a more posterior level. A more intimate approximation of the eyes is to be seen in figure 3. Here the eyes already are con-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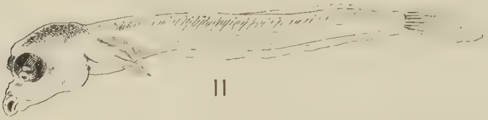
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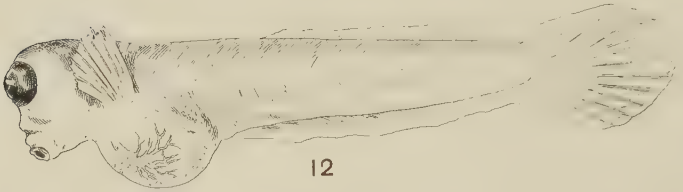
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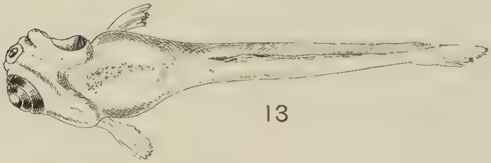
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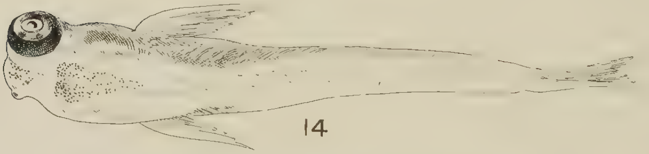
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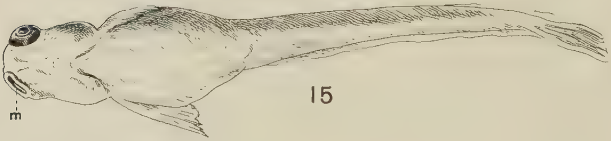

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Fig. 10 Normal Fundulus embryo, eighteen days old, five days after hatching.

Fig. 11 Synophthalmia bilentica, from $\frac{1}{12}$ gram molec. butyric acid, thirty-four days old, three days after hatching.

Fig. 12 Cyclopia synophthalmica, from acetone solution (35 cc. gram molec. to 50 cc. sea-water) twenty-nine days old, one day after hatching.

tiguous medially, but not fused. On microscopic examination it was found, however, that there is a fusion of the median parts of the eyes which becomes the more distinct the more posterior the section examined.

In the embryo presented in figure 4 one median composite eye is to be seen, the components facing each other and enclosing one lens.

Cases of cyclopia, i.e. embryos in which the single median eyes on examination in toto do not present any evidence of being composite in character are presented in figures 5 to 9 and 12. A comparison of these figures shows that the cyclopean eye may vary considerably in size as well as in other respects. It may be very large, much larger than a normal eye (fig. 5) or of about the size of the latter or even smaller, and sometimes, indeed, very minute (fig. 8). Furthermore, the cyclopean eye may have the appearance of a normal eye, or it may vary in that respect. Thus, for instance in figure 9 is seen an embryo with a cyclopean eye lacking a lens, but instead showing a very distinct ventral duplicature of the pigment layer, which was verified on microscopic examination. The ectoderm above the cyclopean optic vesicle was probably defective and the optic vesicle has evidently come into too close contact with the yolk, hence the lack of a lens and the duplicature of a part of the wall of the optic cup. Or again, in figure 6 the cyclopean eye of the embryo exhibits a very striking peculiarity of another kind. Here the eye is seen to be irregular in form, anteriorly it lacks the pupil and is entirely surrounded by the pigment layer, while the lens is seen to be on the postero-lateral aspect of the eye.

Besides the synophthalmic or cyclopean defect I have frequently found embryos with a single eye in the usual lateral position of the head (figs. 14 to 17) and of apparently normal

Fig. 13 Embryo with unilaterally defective head, one normal and one rudimentary eye, from acetone solution (35 cc. gram molec. to 50 cc. sea-water) eighteen days old.

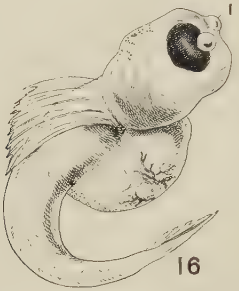
Fig. 14 Monophthalmia asymmetrica, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), nineteen days old.

Fig. 15 Monophthalmia asymmetrica, from acetone solution, (35 cc. gram molec. to 50 cc. sea-water) nineteen days old, *m.*, mouth.

structure. As a serial forerunner of this type of monstrosity may be considered embryos in which one eye is normal while the other is rudimentary (fig. 13).

As illustrative examples of variation in the degree of the eye defects may further be presented some cases in which both eyes are of unusually small size (microphthalmic) and often located on the dorsal side of the head (figs. 18 to 21).

b. Defects of the mouth. It is a well known fact that in the cyclopean or synophthalmic embryos of man and other mammals the nose is almost invariably abnormal in shape, structure and position. It has the form of a proboscis, the nasal passages are more or less blended, a rudimentary septum sometimes being present, while it often may be lacking. Its skeletal parts, if present, consist of cartilage. It is usually situated in the forehead where it hangs down over the cyclopean eye.



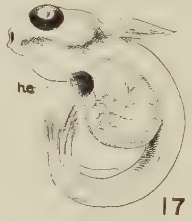


Fig. 16 Embryo with one lateral, malformed eye with free lens, *l*. on eyeless side, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), hatched prematurely on twenty-fourth day after fertilization.

Fig. 17 Embryo with one normal and one heterotopic eye, h. e., from acetone solution (25 cc. gram molec. to 50 cc. sea-water), hatched prematurely on twenty-eighth day after fertilization.

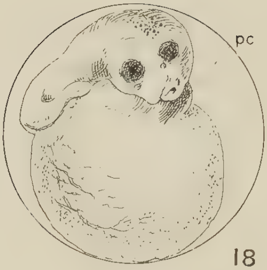
In many teratophthalmic embryos of my experiments a very similar deformity is exhibited by the mouth. The malformation is an unusually striking one. The mouth in the normal embryo (figs. 1 and 10) is broad and flattened and antero-median in relation to the eyes which are situated laterally in the head. In synophthalmic and cyclopean (figs. 2, 3, and 12) and sometimes also in asymmetrically monophthalmic embryos the mouth has the appearance of an elongated snout or proboscis-like structure. On sections of such embryos it was found that this 'proboscis'-mouth is apparently capable of functioning, for the continuity of the oral cavity with the pharynx and oesophagus is nowhere interrupted.

Stockard ('09) who has also obtained this abnormality of the mouth in cyclopean embryos with magnesium chloride in the same material suggests that

> this condition is due to the fact that the single antero-median eye occupies the position normally assumed by the mouth and thus obstructs the usual forward growth of its structures. The mouth, therefore, remains ventro-posterior to the eye and grows downward, presenting the proboscis-like appearance.

This interpretation of the 'proboscis'-mouth as secondary to the 'cyclopean' condition does not appear to be justified, for I have recorded the occurrence of this anomaly of the mouth not only in cyclopean and synophthalmic, but also in asymmetrically monophthalmic and in some two-eyed microphthalmic embryos (fig. 21). In neither of such cases can the eye or eyes be said to occupy the position which is normally taken by the mouth. Moreover, the very opposite may occasionally be found to occur, viz., that in some asymmetrically monophthalmic embryos (fig. 15) the mouth may take the place which should normally be assumed by the lacking eye. There is good reason to believe that the 'proboscis'-mouth results from approximation and partial fusion of the potential anlagen of the maxillary and mandibular arches, following an injury which has destroyed intermediate parts. This becomes strikingly evident on examination of sections.

Our view is further strengthened by Spemann's ('04) findings who, by constricting amphibian eggs, has produced ophthalmic deformities and the 'proboscis'-shaped mouth in the same embryos. In Spemann's experiments the destruction of material intermediate between the potential eyes, the potential maxillary and mandibular arches could not, by any means, be doubted, for here the relation between a well defined mechanical injury and the resulting morphological defect is evident. Moreover, on exami-



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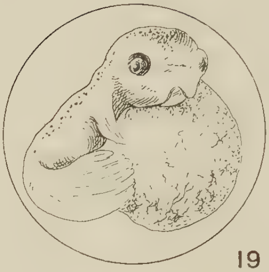

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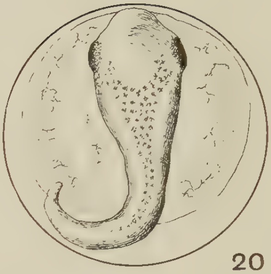

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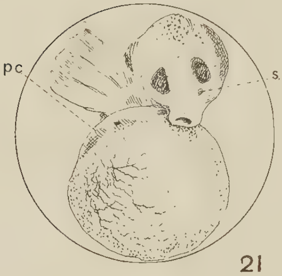

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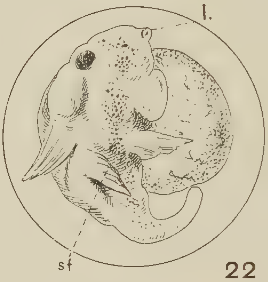

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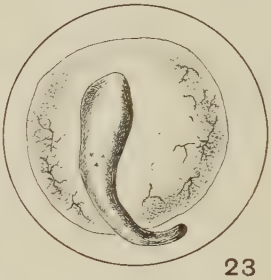

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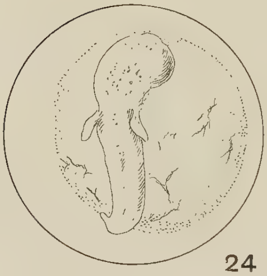

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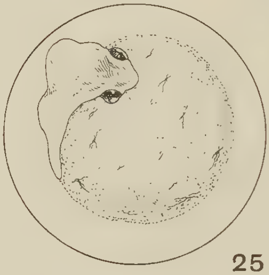

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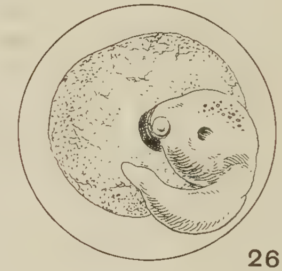

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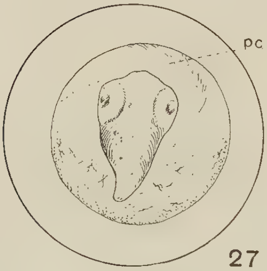

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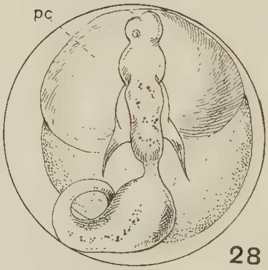

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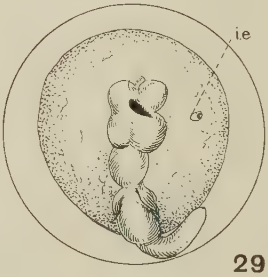

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nation of sections of his monsters Spemann ('04, p. 433) finds that the 'Kieferbogenfortsätze' are "*in der Mitte zu einem verschmolzen.*"[4] That the defects of the mouth are syngenetic with those of the eyes is well suggested also by such embryos where the eyes are small and in a very imperfect condition and the mouth exhibits an anomaly analogous to the 'hare lip' or 'cleft palate' of man (figs. 18 and 19).

This syngenesis of defects of the head is further suggested by the

c. Defects of the olfactory pits. The condition of these organs in teratophthalmic embryos is strikingly similar to the defects exhibited by the eyes. All degrees of approximation and blend-

Fig. 18 Microphthalmic embryo with 'hare lip,' from acetone solution (35 cc. gram molecular to 50 cc. sea-water), thirty-one days old, *p.c.* pericardial vesicle.

Fig. 19 Like 18, twenty-four days old.

Fig. 20 Microphthalmic embryo without fins, with club-tail, from acetone solution (40 cc. gram molec. to 50 cc. sea-water), thirteen days old.

Fig. 21 Microphthalmic embryo, with 'proboscis'-shaped mouth, supernumerary lens, *s.l.*, and one pectoral fin only, *p.c.*, pericardial vesicle.

Fig. 22 Embryo with one lateral rudimentary eye, free lens, *l.* on eyeless side, oedamatous ear vesicles and a supernumerary pectoral fin, *s.f.*, from acetone solution (40 cc. gram molec. to 50 cc. sea-water), thirty-four days old.

Fig. 23 Anophthalmic embryo without fins and with club-tail, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), twelve days old.

Fig. 24 Anophthalmic, malformed embryo with rudimentary pectoral fins and club-tail, from acetone solution (20 cc. gram molec. to 50 cc. sea-water), fourteen days old.

Fig. 25 Greatly malformed embryo, with small eyes, without fins, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), twelve days old.

Fig. 26 Amorphous, oedematous embryo, with rudimentary eyes, from acetone solution (40 cc. gram molec. to 50 cc. sea-water), fourteen days old.

Fig. 27 Deformed embryo, with short posterior part of body and rudimentary eyes, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), thirteen days old *p.c.*, pericardial vesicle.

Fig. 28 Greatly malformed, elongate embryo with waist-like constrictions, with one vestigial eye and club-tail, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), thirteen days old, *p.c.* pericardial vesicle.

Fig. 29 Greatly malformed embryo, with waist-like constrictions, one median rudimentary eye, rudimentary pectoral fins, club-tail and a very small isolated eye, *i.e.*, on yolk-sac, from acetone solution (40 cc. gram molec. acetone to 50 cc. sea-water), thirteen days old.

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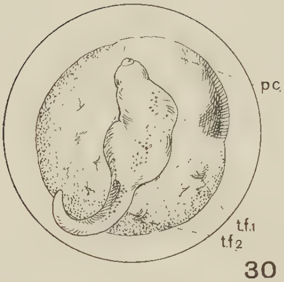
[4] My own italics.



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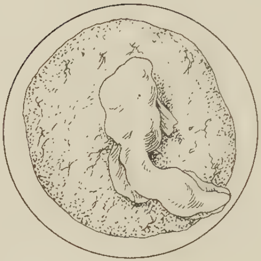

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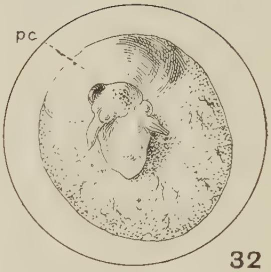

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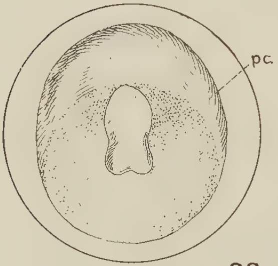

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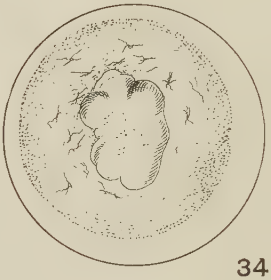

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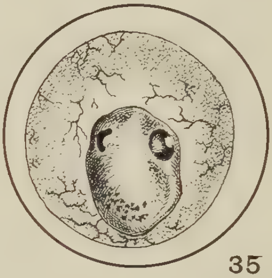

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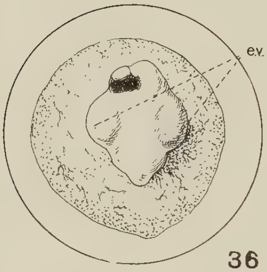

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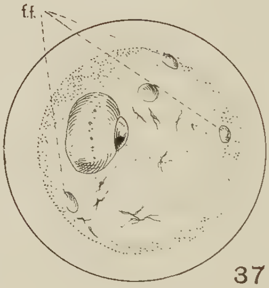

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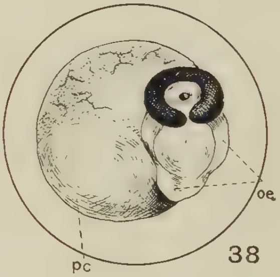

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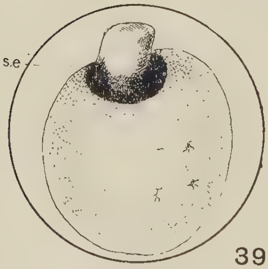

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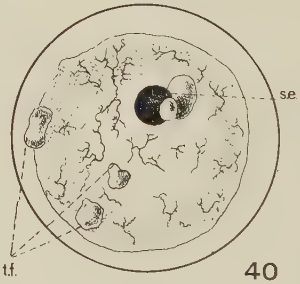

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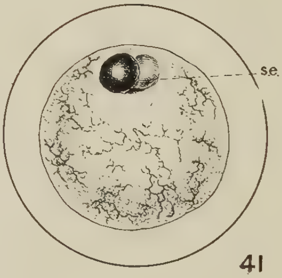

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ing of both pits can be found in various synophthalmic and cyclopean embryos. This observation was made also by Stockard on his 'magnesium-embryos,' but he has not drawn the conclusion to which the data would seem to point. In a subsequent section of this paper dealing with the microscopic anatomy of some terata the attempt is made to account for the teratophthalmic condition, the anomalies of the mouth and olfactory organs on the basis of a morphogenetic factor common to all of these as well as to other deformities in the head region.

d. Defects of the ear vesicles. The deformities to which this organ is subject under the influence of the toxic solutions employed in my experiments, vary considerably. Already on examination in toto of most teratophthalmic or otherwise deformed embryos it can be seen (figs. 2, 6, 8, 22) that the ear vesicles are greatly distended and sometimes reach enormous size. In some embryos there may occur a fusion of both ear vesicles, a condi-

Fig. 30 Amorphous, anophthalmic embryo, with two isolated tissue fragments, $t.f_1$. and $t.f_2$., on yolk-sac; from acetone solution (35 cc. gram molec. to 50 cc. sea-water), thirteen days old, *p.c.*, pericardial vesicle.

Fig. 31 Amorphous, anophthalmic embryo from acetone solution (40 cc. gram molec. to 50 cc. sea-water), fourteen days old.

Fig. 32 Dwarfed, malformed embryo with one lateral rudimentary eye, from $\frac{1}{12}$ gram molec. butyric acid, eighteen days old.

Fig. 33 Amorphous embryo, from acetone solution (40 cc. gram molec. to 50 cc. sea-water), fifteen days old.

Fig. 34 Amorphous embryo from acetone solution (40 cc. gram molec. to 50 cc. sea-water), fourteen days old.

Fig. 35 Meroplastic embryo with rudimentary eyes, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), eighteen days old.

Fig. 36 Meroplastic embryo with one rudimentary lateral eye and oedematous ear-vesicles, *e.v.*, from $\frac{1}{12}$ gram molec. butyric acid, eighteen days old.

Fig. 37 Head-meroplast with rudimentary eye; four small tissue fragments, *t.f.*, on yolk, from acetone solution (40 cc. gram molec. to 50 cc. sea-water) fourteen days old.

Fig. 38 Egg with oedematous eye-teratoma ('solitary eye'), *oe.*, oedema of the brain fragment; from acetone solution (35 cc. gram molec. to 50 cc. sea-water), thirteen days old.

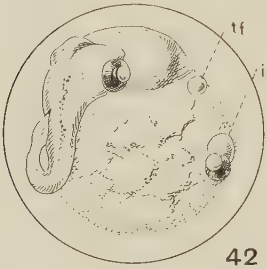
Fig. 39 Egg with eye-teratoma (solitary synophthalmia), *s.e.*, from acetone solution (40 cc. gram molec. to 50 cc. sea-water), thirteen days old.

Fig. 40 Egg with 'solitary eye' *s.e.*, and three blastolytic tissue fragments *t.f.*, from acetone solution (35 cc. gram molec. to 50 cc. sea-water), twelve days old.

Fig. 41 Egg with eye-teratoma ('solitary eye'), from acetone solution (35 cc. gram molec. to 50 cc. sea-water) twelve days old.

tion known in human teratology as synotia. Not infrequently, however, the ear vesicles may be unusually small, and in such cases on microscopic examination it may be found that the semicircular canals are very defective, rudimentary or diminutive in size or that one or two of them may be lacking altogether.

Not many embryos could be tested for their capacity of maintaining the equilibrium while moving about, since few of them would hatch if the eggs were treated with butyric acid or acetone. However, upon several of them, which did hatch, the observation was made that they could swim only in circular or spiral lines, or along the wall of the fingerbowl in which they were kept, while they could not move in a straight forward direction, dropping at once to the bottom of the dish, if forced to do so. This functional anomaly agrees well with the structural defects of the semicircular canals spoken of above.



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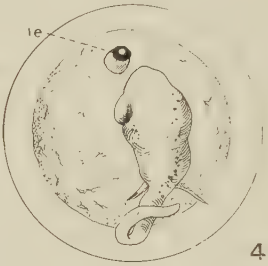

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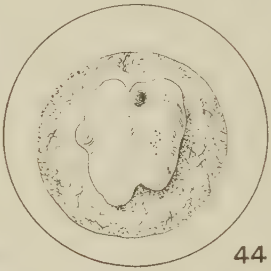

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Fig. 42 Asymmetrically monophthalmic embryo, with club-tail, without pectoral fins. On the yolk-sac at a distance from the embryo is seen an isolated tissue fragment *t.f.*, and an isolated eye *i.e.*, from acetone solution (40 cc. gram molec. to 50 cc. sea-water), twelve days old.

Fig. 43 Asymmetrically monophthalmic embryo with isolated eye, *i.e.*, from acetone solution (25 cc. gram molec. to 50 cc. sea-water), sixteen days old.

Fig. 44 Amorphous embryo, in toto making the impression of malformed coalesced twins, from acetone solution (25 cc. gram molec. to 50 cc. sea-water), twelve days old.

2. The amorphous embryos

This group extends over a wide range of monstrous embryos. It begins with forms which on examination in toto in their shape, size and structural peculiarities only faintly suggest the resemblance with fish embryos of a corresponding age, and goes all the way down to such forms where such gross-morphological similarity is entirely obliterated (figs. 24 to 34).

Thus embryos may be found with rudimentary eyes in which the anterior part of the body has widened out to a very striking degree, with club-fins and a club-tail. Not uncommon is the occurrence of greatly elongated and misshapen embryos often with waistlike constrictions (figs. 28 and 29), with rudimentary fins or (sometimes) dislocated fins (figs. 29 and 31). This group would comprise also some remarkably misshapen and dwarfed embryos (figs. 25, 26, 27 and 32) and finally embryos whose form would almost seem to suggest a similarity to some invertebrate animals (figs. 33 and 34).

3. Meroplastic embryos

Very great numbers of eggs were found both in butyric acid and acetone experiments in which parts of the bodies have developed, while the rest of the germ has apparently suffered destruction ('Meroplasts'-Roux, l. c.).

In such meroplastic embryos the same wide range of variation obtains in the degree of defect, as in all other monsters recorded above. Common to nearly all of them, however, is that, usually it is the anterior part of the body that develops, while the posterior part is lacking. The variations in defect concern the organs of the meroplasts as well, as the quantity of what has developed. A glance at figures 35 and 36 shows that the eyes may be variously defective and the same obtains for the ear-vesicles. Likewise the shape and size of the meroplasts are very inconstant. The meroplastic embryo may correspond to more than the anterior half of an embryo or be just about half of the embryo's body. The latter ones recall the hemiembryos which Roux ('95) and later other investigators have obtained by injuring one of the first two blastomeres of the frog's egg.

Finally the meroplasts should be mentioned which are less than half of the body (figs. 37 to 41). Not infrequently all that can be seen to have developed is a more or less malformed head, recognized only by the presence of a rudimentary eye (fig. 37).

Even smaller, usually amorphous, meroplasts can be found in some eggs as the only evidence of development. As the most significant of all the meroplasts recorded may be regarded eggs in which nothing could be found on the yolk-sac besides a very small tissue fragment with an eye ('solitary eye') (figs. 38 to 41). The morphology of such ova and their ontomechanical significance will be discussed under a subsequent heading.

* * *

Of other deformities recorded in these experiments several double monsters would seem to deserve description. Owing, however, to limitations of space, the presentation of these observations is reserved for a paper (soon to be published) which is to deal with a rather large number of various duplicities recorded in more recent experiments.

III. THE MICROSCOPIC ANATOMY OF THE MONSTERS AND CONCLUSIONS REGARDING THEIR MORPHOGENESIS

This part of the work has yielded some very encouraging results. It has, as I hope to make clear, contributed not only a basis for rational interpretation of the morphogenesis of some monsters, but also disclosed some general principles which may underly the genesis of all malformations recorded in my experiments, and possibly of such as occur spontaneously in a state of nature. While for this reason an extensive study of these terata would seem very desirable, I have so far not been able to study more than a few common types. A more extensive treatment of these experimental terata will follow this communication in further studies.

In the present study I have given most attention to the morphology of ophthalmic terata.

A. CLASSIFICATION OF OPHTHALMIC TERATA

There being, as I have mentioned before, a very great variety of these deformities, a classification of them, although of necessity arbitrary to some extent, would seem desirable. For, it is obvious that the term 'cyclopia' in its present use does not define any one of the conditions which it is meant to cover. A similar objection must be made to the term 'semi-cyclopia' (Gemmill, '12) for, the terata, which this term would define, are of many kinds, and while gross-morphologically similar, they mostly differ in important structural detail. It is also hardly necessary to emphasize that the term 'hour-glass eye,' so commonly employed, is wholly unscientific, for it utterly lacks the precision that is necessary to define a morphological condition.

Fairly serviceable classifications of the various eye monsters have been made by Vrolick ('49), Kundrat ('82) and Bock ('89). However, these classifications being based on mammalian terata and considering not only defects of the eye but also the correlated defects of the nose and skull are lacking in precision (even if applied to mammals only), and do not well lend themselves to other vertebrates. A rational classification which would consider the deformities of both these organs appears to be well-nigh impossible.

I have, therefore, attempted the following simple classification of the teratophthalmic ('cyclopean') monsters with the condition of single- or two-eyedness respectively as the only and basic morphological criterion:

- I. Synophthalmia
  - a. bilentica
  - b. unilentica.
- II. Cyclopia s. Monophthalmia mediana
  - a. synophthalmica.
  - b. perfecta.
- III. Monophthalmia asymmetrica (s. lateralis)

Under 'synophthalmia' will be classified cases of either more or less approximated, closely approximated eyes, or eyes so fused that the composite character of the organ is easily discernible in toto. If such an optic organ should possess two symmetrically placed lenses, the term 'synophthalmia bilentica' will be applied, while, if on more intimate fusion of the eye components only one lens should be present, 'synophthalmia unilentica' will be used as the descriptive term.

The term 'cyclopia' will be applied where a single median eye is present, which on examination in toto does not present the appearance of a composite eye. If, however, such an eye on microscopic examination of sections is found to consist of two blended eye components, I shall term it 'cyclopia synophthalmica.' To this category belong by far the greatest number of cyclopean monsters.

Of rare occurrence in my experiments (as well as in the cases of spontaneous cyclopia described by various authors) were found to be cyclopean eyes which on microscopic examination were single throughout and nowhere suggesting the possibility of their being composite in character. Such cases I shall henceforth term 'perfect ('true') cyclopia' (Schwalbe's 'cyclopia completa'). In perfect cyclopia the eye is, as a rule, more defective than in synophthalmic cyclopia. It may be very small in size (microphthalmic) or some of its structures may be more or less defective or even lacking entirely. In perfect cyclopia the defects of the brain and other defects are usually much greater than in synophthalmic cyclopia.

The term 'Monophthalmia asymmetrica' has been in use since its introduction by Ahlfeld ('80–'82) and would seem to need no further comments. The embryos of this group possess one eye in the usual lateral position of the head.

B. THE MORPHOLOGY OF TERATOPHTHALMIA

A fairly extensive study of teratophthalmic embryos in sections has enabled me to make many observations which directly or indirectly point to certain dynamic factors underlying their formation. It was found that these embryos sustain at an early stage of development an injury mainly in a restricted area of the anterior end of the future embryo's body which eventually leads to the formation of the terata of the eye. In the following I shall now present anatomical descriptions of various types of teratophthalmia and such evidence will be pointed out as may reasonably be adduced to the interpretation of their morphogenesis. Our description will begin with bilentic synophthalmia, where the defect is yet relatively slight and take up successively the more extreme malformations of the 'cyclocephalic group' through cyclopia all the way down to anophthalmia. The anatomy of asymmetric monophthalmia will also be considered, which, while being outside of the 'cyclocephalic group,' yet exhibits ample evidence that it owes its formation to like morphogenetic factors.

a. Synophthalmia bilentica. One of the embryos which we have chosen for the presentation of this malformation is seen in toto in figure 3 (p. 488). The eyes are large, not fused, but very closely adjacent. On microscopic examination it is seen that in anterior sections the eyes are separate (fig. 63). If, however, the whole series be examined, it is found that at about the level at which the lens appears in the sections, the medial margins of the eye-bulbs begin to blend; and when followed more posteriorwards this fusion becomes so intimate as to manifest itself in a unition of the retina of one eye with that of the other. The eyes appear to be otherwise normal in structure and two optic nerves are present which are seen to enter the optic lobes after having formed a chiasma (fig. 64).

Examination of the entire series of sections of this embryo suggests that the injury sustained by it which is responsible for its ophthalmic malformation was apparently restricted to the most anterior part of the future embryo's body. This is evidenced by the following data. The abnormalities such as are found to characterize this embryo concern the mouth, the olfactory organ and the most anterior part of the brain, viz., the fore-brain. As seen in figure 3 the mouth is a typical proboscis, and the olfactory pits are in sections seen to be perfectly blended into one large pit (fig. 63). The fore-brain is abnormal in structure and unpaired (fig. 63), while the mid- and hind-brain are bilaterally symmetrical and apparently normal in other respects (fig. 64). On following out the whole series of sections no other abnormality can be detected. The injury sustained is thus very clearly seen to be restricted to the embryo's anterior end. Its probable nature and its morphogenetic consequences will be pointed out in the course of the following description of other embryos where a like teratogenetic principle seems to obtain.

b. Synophthalmia unilentica. The embryo selected for the description of this deformity is from the same experiment as the preceding one and was twenty-four days old when killed. In toto (fig. 4) it almost made the appearance of a perfectly cyclopean monster, there being only a single median eye present with one centrally located lens. The pigment wall of the eye-bulb, however, was strikingly abnormal, because presenting a figure similar to two C's blended at their opposite ends, it indicated the composition of the eye of the ophthalmoblastic materials of both sides.

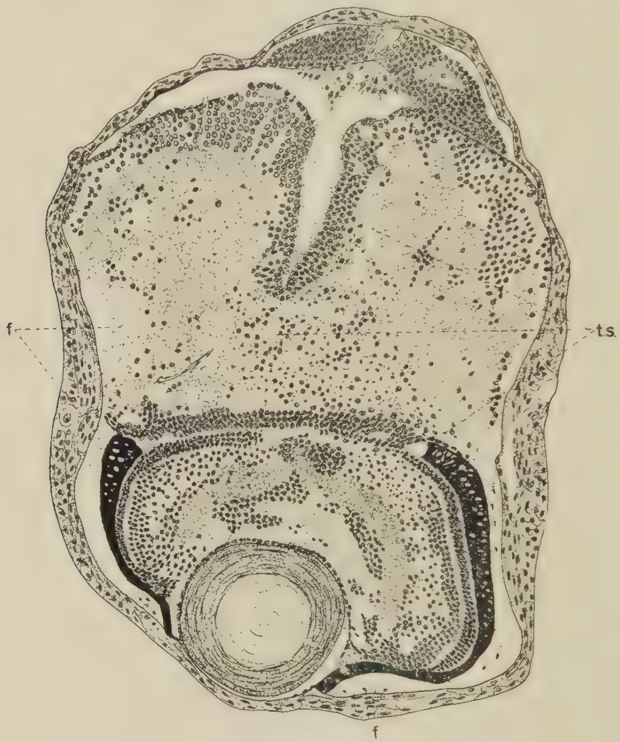

Fig. 45 Camera lucida drawing of a transverse section through the eye of the embryo in figure 4. *t.s.*, tissue spaces; *f.*, fibrin. × 173⅓

On microscopic examination of this embryo the following findings were noted. The eye is composed of two incomplete optic cups facing each other and enclosing a single lens of about the usual size (fig. 45). The cornea and iris are apparently normally developed. The anterior chamber of the eye is absent and the vitreous body only barely suggested. The retina, while being well differentiated, is abnormal in some of its layers, which are to a considerable extent intermingled with the fibrous layer. Some of the retinal layers of the two optic cups are continuous in sections through the anterior part of the eye (fig. 45) while no such continuity can be observed more posteriorly (fig. 46). Two optic nerves are seen to pass out of the eye in few and loose

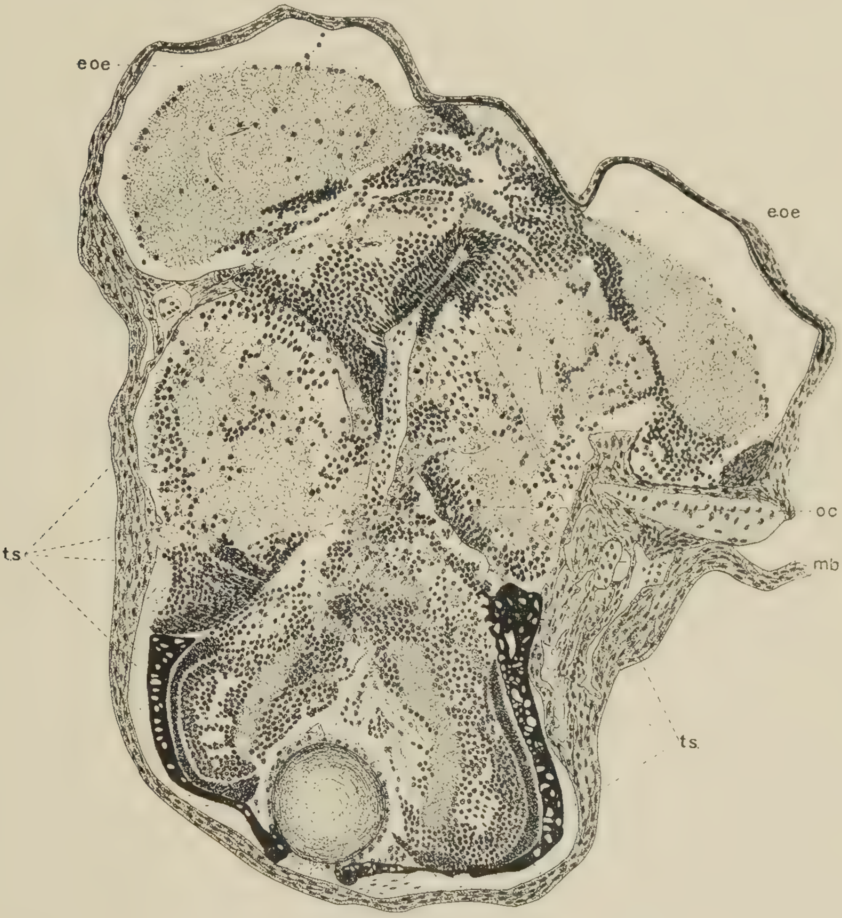

Fig. 46 Camera lucida drawing of a more posterior section through the eye of the same embryo (region of optic lobes). *t.s.*, tissue spaces; *e.oe.*, extracerebral oedema; *o.c.*, optic cross, *mb.*, mandible. × 173⅓.

bundles of fibers and to enter the opposite sides of the brain after having formed an indistinct cross (fig. 46).

The incompleteness of the fused optic cups and the other abnormalities exhibited by this eye are probably due to the circumstance that at a very early stage of development a large part of the ophthalmoblastic material had undergone destructive changes and has suffered partial elimination owing to the chemical action of the toxic solution and physical action (increased osmotic pressure) due to subsequent transfer to pure sea-water. The injury sustained by the embryo has apparently been the severest at the most anterior point of the chief body axis, diminishing gradually posteriorly. The following data would seem to substantiate this interpretation. The malformation is restricted mainly to the eyes and the anterior part of the head. For, examination of sections shows that the fore-brain of this embryo is unpaired and otherwise defective, while the rest of the brain is, when followed in sections posteriorwards, seen to gradually present more and more distinctly the condition of bilateral symmetry. The mid-brain and hind-brain while being bilateral, exhibit, however, a certain other abnormality. The injury here was apparently restricted to the blood and lymph vessels, the earliest anlagen of which seem to have been arrested in their development. This condition can be recognized by the great number of large, clear and empty spaces (figs. 45 and 46) in the tissues of the posterior parts of the brain which in the living embryo have apparently been filled with fluid owing to the existing imperfection in the circulation. A condition of oedema has thus apparently resulted from lack of drainage, an analogue of which is also represented by the oedematous distension of the cranial cavity in the region of the fore-brain and mid-brain. No other abnormalities of the posterior parts of the brain or any other part of the embryo can be noted, which suggests the conclusion that the anterior part of the embryo's body is the most sensitive one and thus subject to the highest degree of injury.

c. Cyclopia synophthalmica. The distinction between this deformity and the preceding one is based on morphological differences existing between the two types of a genetically similar malformation. These differences are apparently such of degree only, and probably due to differences in the degree of intensity of action of the same morphogenetic factors.

Synophthalmic cyclopia is characterized by the presence in the embryo of a single median eye usually of a larger size than the normal eye, not composite in its appearance in toto but very markedly so on microscopic examination of sections. The following two cases may illustrate this deformity.

In figure 5 (p. 488) is presented an embryo which has a single, very large, well-formed, median eye, which on examination in toto reveals nothing that would suggest its composite character.

On microscopic examination of transverse sections the most anterior sections still present the appearance of a solid non-composite eye, while at about the level at which the lens begins to appear in the sections, the eye-cup discloses its composite character the more the further the sections are followed out posteriorwards, until at the level of the optic lobes this condition of fusion is seen to be very striking. In this region, as well as somewhat anterior and posterior to it, the optic cup presents the appearance of the horizontal section of a funnel (fig. 65). The small end of this funnel is blind and enclosed by the brain, into the substance of which it is seen to dip to a remarkable depth. This funnel-shaped eye is unusually large and the brain is strikingly small. The structures of the optic cup are well differentiated as far as the large part of the 'funnel' is concerned, for here the pigment layer and all layers of the retina are present in their typical appearance. In the small end, however, there are only slight traces of the rods-and-cones layer between the outer margins of the 'funnel' and the brain, while of the other parts of the retina the fibrous and ganglionic layers are present throughout and are seen to be in continuation with these parts of the large end.

The interpretation of the morphogenesis of this cyclopean eye is facilitated by examination of sections of the entire head. The following conditions are revealed by it. The olfactory pits are fused. The fore-brain is unusually short and unpaired, while the mid-brain is bilaterally symmetrical but not distinctly divided into two hemispheres. This is very striking on comparison of figure 64 with figure 65. Both illustrate sections of approximately the same region. While in the former (the case of bilentic synophthalmia described above) the separation of the two hemispheres is very distinct, in this cyclopean embryo (fig. 65) there is an apparent rupture of the solid brain mass caused evidently by the expansive growth of the eye which it tightly encloses. This is a break rather than a natural division and it can be followed throughout the entire mid-brain, while the hind-brain is distinctly bilobed. No other abnormalities were found in this embryo.

These data suggest that the ovum at an early stage of its development has sustained an injury at the anterior end of the future embryo's chief body axis. The injury apparently consisted in a destructive elimination of a small, very sharply pointed wedge of tissue (the point directed posteriorwards), comprising the future interocular area, possibly a small part of the opthalmoblastic material, and, evidently, also a considerable part of the future brain. The coalescence of the wound surfaces has caused an approximation and subsequent fusion of parts, which in turn eventually resulted in cyclopia.

The other case of synophthalmic cyclopia concerns an embryo (fig. 12) (p. 490) whose head exhibits some striking features. It is relatively very small (microcephalus), the mouth is a wide open, typical proboscis and the eye is single, median in position, very large and betrays on examination in toto no evidence whatever of being composite in character.

In anterior sections of this embryo (fig. 66) the appearance of a normal, transversely sectioned, eye is presented, and the synophthalmic character is revealed only by examination of sections at a more posterior level. However, if the brain is examined in anterior sections the nature of the process is disclosed to which the malformation secondarily owes its origin. The forebrain is unpaired and very small, for, in size it hardly exceeds that of the eye. The most remarkable feature, however, which the brain at this level presents, is a fragment of retina which is fused with it at its lower extreme right and just above the orbit of the eye. The significance of this retinal fragment is at once recognized when the condition of the whole eye is considered. On following the sections posteriorwards the eye more and more appears to be oval in shape. More posteriorly yet, the eye widens out enormously (fig. 67) while the brain is at this point very distorted and strikingly small in size. A few sections further posteriorly, the shape of the eye is still practically the same, but its size has diminished somewhat while that of the brain has increased. The latter which is now in the region of the optic lobes is very distinctly bilobed, the right hemisphere, although somewhat distorted, is, however, complete, while of the left one about a half is wanting, the place of this lacking part of the brain being occupied by the larger part of the eye, which at this level is horse-shoe-shaped in cross-section. The very last sections of the eye prove unmistakably that it is composite, for its base consists of two separate optic cups.

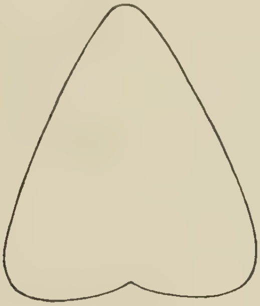

Fig. 47 Diagrammatic outline reconstruction of the cyclopean eye of the embryo in figure 12.

The nature of this eye is best understood from the diagrammatic outline reconstruction which is attempted in figure 47. It is a heart-shaped body with the apex directed frontalwards and the base cerebralwards.

The question now arises in what way this peculiar malformation came about. The intracerebral retinal fragment which was referred to above (fig. 66) as well as the shape of the eye and the defects of the brain point to an answer which, I think, contains a high degree of probability. Here, too, the injury sustained by the embryo was restricted to its most anterior portion. Owing to this lesion resulting from a process of destruction which I term blastolysis, a wedge of blastema (with the sharp point directed posteriorwards) was eliminated, and the subsequent coalescence of the wound surfaces has caused the earliest optic anlagen to fuse. The eliminated wedge-shaped piece of tissue contained apparently the future interocular area, much more of the anterior parts of the ophthalmoblastic material of both sides than of their posterior parts, and a part of the potential brain. This would account for the perfect fusion of the anterior part of the eye components, as well as for the incomplete fusion of their posterior parts, of which probably very little had been lost. That the process which caused the injury was apparently one of dissociation and dispersion (blastolysis) would seem to be evidenced by the retinal fragment, which can only secondarily have come to fuse with the brain, i.e., after the ophthalmoblastic fragment which has given rise to it had been dislocated cerebralwards from its natural position.

d. Cyclopia perfecta. This monstrostity represents a very high degree of ophthalmic malformation. In its morphogenesis it differs from synophthalmic cyclopia, for the perfectly cyclopean eye is genetically a single eye. However, the evidence which I have been able to find, points to the same dynamic process, namely, blastolytic action of the altered environment as the factor responsible for its formation.

A few examples may now be presented.

In figure 6 (p. 488) is presented an embryo which is possessed of a single median eye and small defective fins, partly obscured from view by the very large oedematous ear vesicles. The whole body is distended and the tail is finless and club-shaped.

The examination of the eye discloses a very remarkable condition. Already in toto (fig. 6) the eye is seen to be abnormal in shape and in the position of the lens, which is situated posterolaterally, instead of being anteriorly and in the center of the eye. The front of the eye is entirely closed over by the pigment layer, so that the condition presented might almost be considered as a rotation of the polar axis of the organ.

The microscopic examination of the sectioned embryo confirms the macroscopic appearance. The whole eye appears in forty-nine sections of 6μ thickness. Up to the twenty-second section no trace of a lens is seen, and in these anterior sections the condition is presented which normally obtains for posterior sections, i.e. the view is one of the base of an eye where it is entirely encircled by the pigment layer. In the section illustrated by figure 68 of Plate 1 the retinal layers are defective and the brain, while being bilobed, yet appears to be greatly distorted. Following the sections posteriorwards we see in the twenty-second section (fig. 69) the beginning of the lens in its abnormal position. It extends up to the forty-seventh section inclusively and it is thus seen to occupy about the posterior nine-sixteenth of the eye. No optic nerve can be found, although the fibrous layer of the retina is fairly well developed. The mouth is absent in this embryo and the pharynx comes into view in the last sections through the eye, which is seen to be partly enclosed by the defective mandibular arches and projecting for the greater part into the distended pericardial vesicle.

The optic cup is C-shaped; it strikingly suggests the similarity to a component of a synophthalmic eye and points to the morphogenesis of this monstrosity. Owing to blastolysis the future interocular area of the early embryo, the entire ophthalmoblastic material of one side and a part of it of the other side were destroyed as well as also the earliest anlage of one olfactory pit. The subsequent approximation of the wound surfaces has moved the remainder of the one uninjured ophthalmic anlage out of its original position so that the incomplete optic cup which has developed from it, has turned at an angle of about 90° in relation to its axis. The apparent heterotopia of the lens and the pigment-enclosed front of the eye are evidently due solely to this secondary change in position of the remaining part of one optic anlage. No traces of a fusion could be found anywhere, and the presence of a single (non-fused) olfactory pit (fig. 68) would seem to strengthen the evidence that the cyclopean eye of this embryo has developed from the ophthalmoblastic material of one side only.

The injury sustained by the embryo was a severe one, much severer than is usually found in either synophthalmia or synophthalmic cyclopia. This is evidenced by the defective and oedematous condition of the brain (fig. 69), and the curving of the head, to which is due the appearance in one section of the eye, medulla, and semicircular canals. Yet the morphogenetic factor which brought about the embryo's deformity was, no doubt, the same as in synophthalmic monsters.

A case of perfect cyclopia, where the malformation is far more extreme than in the preceding one is presented in figure 8 (p. 488). The embryo is seen to be extremely deformed. It has a single, median, unusually small eye, and greatly distended ear vesicles (cf. fig. 71); the entire body is oedematous, all fins are lacking and the tail is club-shaped.

On microscopic examination (fig. 70) the eye is seen to be genetically single, there being no indication whatever of its having been formed out of optic anlagen of both sides. It is very rudimentary in structure, the pigment layer and the relatively very large lens being its best developed parts, while the retina is very defective, the optic nerve, the iris, anterior chamber and the vitreous body lacking altogether. The brain is lateral to the optic cup of this minute eye instead of being dorsal as should be expected from the position of a cyclopean eye. This distortion of the relation between the eye and the brain, the defective and unpaired condition of the latter throughout (cf. fig. 70) and the deformities of the rest of the body suggest that a severe injury was sustained by the entire embryo. However, the defects are most extreme at the embryo's anterior end, which again points to the conclusion that in this case, too, the degree of injury was the highest at the most anterior part of the early embryonic anlage, diminishing posteriorly along the chief body axis. The eye arose from a fragment of one potential optic anlage, the remainder of which and the entire other potential optic anlage as well as a part of the future brain and the potential olfactory pits having suffered destruction. Owing to subsequent processes of regulation the surviving ophthalmoblastic fragment has come to occupy the median position in the defective head where it developed into the rudimentary eye.

One more case of perfect cyclopia may now be described. On examination of the embryo in toto (fig. 7, p. 488) it was seen that the cyclopean eye is lacking the lens. This may be attributed to the circumstance that the optic vesicle came into contact with damaged head ectoderm.

On examination of sections (fig. 48) conditions are found which again point to blastolysis (dissociation and dispersion) and subsequent regulation as the factors responsible for the formation of perfect cyclopia. The retina is defective, its rods-and-cones layer being fairly well developed on one side and less so on the other side of the optic cup. The ganglionic granular and fibrous layers appear to be scattered and intermingled. Several insular accumulations of retinal cells surrounding fibers can be observed resembling the 'retinal rosettes' recently described by Nehl ('14). The oedema evidenced by numerous large tissue spaces in the eye and in the brain (which latter is highly defective and unpaired), and the distension of the cranial cavity are very likely due secondarily to blastolytic action, the blood vessels, owing to destruction of embryonic material, having failed to develop into a continuous system of drainage. Nothing can be seen in any of the sections that would indicate a fusion of two optic anlagen. Only one optic nerve is seen to pass out of the eye and enter the brain. The complete absence of the olfactory pits and the mouth (the latter coming into view in sections behind the eye) also strengthens the evidence for blastolytic action (dissociation and dispersion) of the environmental modification employed in the experiment. Owing to this action, evidently, the ophthalmoblastic material of one side has been destroyed, while that of the other side has, through subsequent reparation, come to occupy a median position.

e. Monophthalmia asymmetrica. I shall now attempt to show that the same morphogenetic factor (blastolysis) is responsible also for the genesis of other cases of teratophthalmia. They are exemplified by embryos in which both eyes are present in the typical later position in the head, one of them being normal, while the other is small in size and rudimentary in structure, by embryos in which one of the eyes is dislocated (ophthalmic ectopia), and by asymmetrically monophthalmic embryos.

The first case now to be described is that of an embryo with one small rudimentary eye, the other eye being apparently normal (fig. 13, p. 490). The embryo is very small as compared with normal embryos of the same age after hatching, its head is curved towards the side of the rudimentary eye, but no other abnormalities could be noticed.

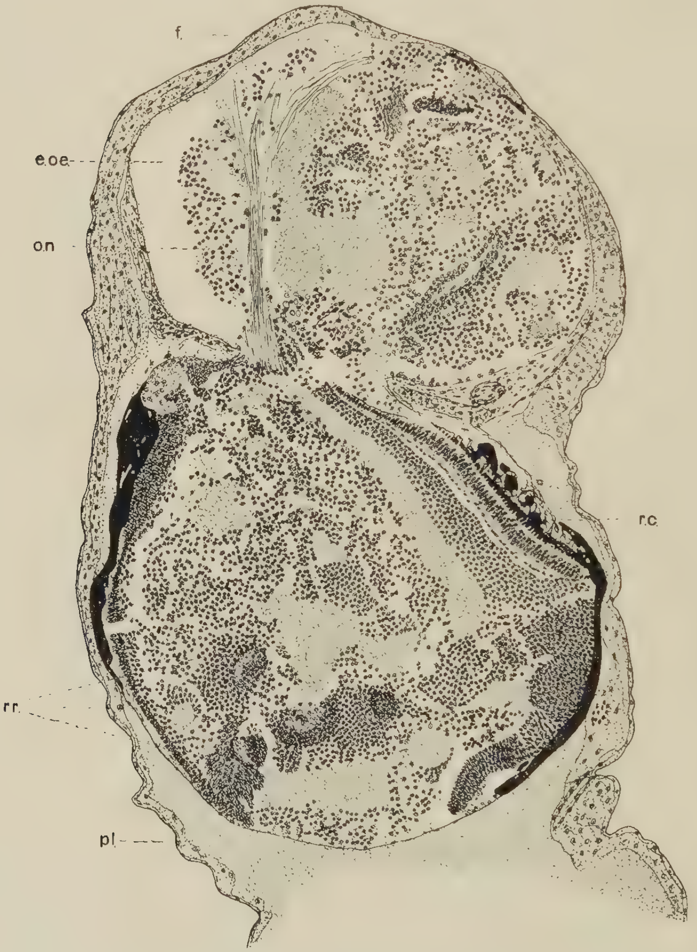

Fig. 48 Camera lucida drawing of a transverse section through the eye region of the perfectly cyclopean embryo in figure 7, *r.c.*, rods and cones; *r.r.*, 'retinal rosettes'; *o.n.*, optic nerve; *f.*, fibrin; *e.oe.*, extracerebral oedema; *pl.*, plasma in pericardium into which the eye dips. × 173$\frac{1}{3}$

In microscopic sections the embryo's right eye appears to be perfectly normal in every respect, while the left eye appears in the sections posterior to it and extending into the yolk-sac, is diminutive in size, but well differentiated in essential structural details. The optic nerve of the small eye is seen entering the brain independently of the same nerve of the other (normal) eye.

On examination of the entire series of sections the probable nature of the injury sustained by the embryo is gradually disclosed. It is restricted mainly to one side and the abnormalities concern the olfactory pit, the eye, the brain, and the ear vesicle.

While the olfactory pit of the uninjured side is normal and in its typical position antero-median to the normal eye, there are three olfactory pits on the abnormal side, all of which are closely approximated (fig. 72). This points to a fragmentation of the potential rhino-ectoderm at a pre-differential stage of development. The condition of the eye of this side has been described; its probable manner of formation is suggested by a careful examination of the brain. The latter in successive sections is found to consist of two hemispheres, but is strikingly asymmetrical in regard to the position occupied by them in relation to the chief body axis, the right hemisphere preceding in all sections the left one which is pushed posteriorwards (fig. 73). This agrees well with the posteriorward dislocation of the eye on the same side and is probably due to regulation after a sustained unilateral lesion. Striking evidence of a process of dissociation (blastolysis) is found on examination of sections at the level of the optic lobe of this side (fig. 74). The latter, which, like the whole hemisphere of this side, is posterior to the one of the right side, is seen to be at the same level with the posterior part of the left (smaller) eye, with the right ear, the heart, and the yolk of the body cavity. It is incomplete, and looks as if a part of it had been broken off. To the right from it (in the figure) there is a wide cleft. Between it and the head integument there is a large fragment of tissue (o. l. f.) which has the appearance of a small optic cup at an early stage of differentiation, but on careful examination of all sections is found at its most ventral point to be in connection with the optic lobe. This tissue fragment must thus be considered as a part of the optic lobe, from which it was for the greater part delaminated. The ear of the left side is lacking entirely, and it stands to reason that its absence is due to blastolytic elimination of the otoblastic material of this side.

Very striking evidence of blastolysis can be observed in the embryo in figure 17 (p. 492). It hatched prematurely on the twenty-eighth day, when it was drawn and killed. The yolk-sac is still very large and the embryo is curved. Only the left eye is in the usual position in the head while the right eye is found to be strangely dislocated. It is seen extending between the right mandibular arch and the yolk, in which it is well imbedded, covered by the yolk-sac.

Examination of sections reveals the following conditions. The left eye is well differentiated, but not quite normal. It lacks the anterior chamber and the vitreous body, while the iris is rudimentary. An optic nerve is present and can be followed out to enter the hemisphere of the opposite side. The brain is apparently normal everywhere. In the sections at the level of the medulla and semicircular canals there comes into view the heterotopic eye (fig. 75). It is enclosed between the yolk and the yolk sac and is in proximity to the heart. Between the heart and the dislocated eye very large lymphocytes are seen. The eye is as well differentiated as the orthotopic left eye and is oval in shape, which may perhaps be due to its extra-orbital development. It lacks a pupil and the vitreous body, while the iris also appears to be defective. No optic nerve could be found.

An important clue to the genesis of these malformations is furnished by the condition of the olfactory pits. The latter can be followed out very satisfactorily in the most anterior sections. Antero-median from the left (orthotopic) eye there is one olfactoroy pit—in its normal position. On the right side, however, (where the eye has been dislocated) there can be seen in sections somewhat more posterior, as many as four minute olfactory pits. It seems obvious that these four small olfactory pits have developed from four fragments of the dissociated potential anlage of the olfactory pit of this side. Dissociation has, then, on this side affected both the potential olfactory and eye anlagen, fragmenting the one and delaminating and dislocating the other. The effect is an increased number of olfactory pits and ophthalmic heterotopia.

The same morphogenetic factor was apparently responsible also for the conditions found in the embryo in figure 16, p. 492. Its head is unusually large and suggests oedema. The left eye is lacking entirely and a free lens is found in its place. The right eye is defective, the optic cup being C-shaped and the large lens greatly protruding, owing to the absence of an anterior chamber.

In the most anterior sections only the 'independent' lens is seen which is not yet fully differentiated. In the next sections there comes into view the lens of the right eye and two olfactory pits. The latter are observed to be so closely approximated as to be partly contingent on their median borders, and to form an angle of about 90 degrees. More posterior sections show that the optic cup of the eye is only anteriorly C-shaped, while it is complete, though small, in its posterior part. The part of the section where the (lacking) left eye should be, is occupied by very loose mesenchyme the interstices of which are filled with plasma. The retina is very well differentiated and one optic nerve can be traced to its entrance into the brain hemisphere of the opposite side. A very remarkable feature is presented by the sections through the base of the eye (fig. 76). Here, ventral to the brain and lateral from the eye from which it is entirely separated, there can be observed a very small optic cup-fragment with all layers (pigment and retinal-rods and cones) perfectly differentiated. This is evidently a dislocated but fully differentiated remnant of the destroyed ophthalmoblastic material of the left side.

Essentially similar conditions were found in the embryo illustrated by figure 15 (p. 490). A ventral view of it is presented in which it can be seen that the right eye is lacking and its place is occupied by the mouth. The left fin is about half the normal size, and no other abnormalities were found on examination of the embryo in toto.

The microscopic examination of sections revealed on the left side (right side in the illustration, fig. 77) a practically normal eye. On the right side in the place of the eye is seen the mouth cavity. The ophthalmoblastic material which was to form the right eye has been largely destroyed and only a small fragment of it has been left, which has developed into an optic cup-fragment, seen enclosed in the cranial cavity at the base of the brain. This fragment of the optic cup shows all layers, including the fibrous layer, of the retina well differentiated.

The brain is bilobed and no abnormalities can be found in it excepting its oblique position in the head with regard to the main body axis. This is either secondary to or syngenetic with the herterotopia of the mouth, which in turn is due to the destruction of the ophthalmoblastic material of one side. The elimination of the latter has allowed the mouth to expand in the direction of least resistance so far, as to occupy the exact position of the lacking eye, while the excessive expansion of the mouth on this side may have caused distortion of the brain in its relation to the body axis.

Very interesting conditions are found also in the embryo, a dorsal view of which is presented in figure 14 (p. 490).

The left eye is lacking and there is a very distinct invagination where the eye should be. On examination of the ventral side the mouth could be seen to be of a shape approaching the 'proboscis' type. Its position was very near to what was to be the place of the lacking eye. No other abnormalities could be observed in the embryo in toto.

Microscopic examination of sections reveals a normal olfactory pit and normal eye on the right side. In sections through the posterior third of the eye (fig. 78) the mouth appears almost exactly in what was to be the place of the eye. An unusually small left olfactory pit comes into view at this level and a minute 'independent' lens is noted on the maxilla. Two more minute lenses are found on sections still more posteriorly (fig. 79). The origin of these lenses on the eyeless side I am inclined to consider as due to contact of remnants of the destroyed ophthalmoblastic material of this side with the ectodermal epithelium.

The brain is bilobed, but asymetric in regard to its relation to the chief body axis, the hemisphere of the side possessing the eye preceding in sections that of the side lacking the eye.

The lesion sustained by the early embryo was evidently restricted to the left side of its anteriormost part, where it has eliminated the entire ophthalmoblastic material, minute remnants of which have apparently stimulated the differentiation of free lenses. Owing to subsequent processes of regulation, the injured side of the head suffered a posteriorward displacement. This would account for the small size and unusual position of the left olfactory pit as well as for the asymmetry in the position of the left hemisphere of the brain.

f. Microphthalmia and Anophthalmia. Microscopic examination of sections of embryos with very small, rudimentary eyes, or such in which no eyes can be detected in toto have likewise disclosed conditions which point to the action of the same dynamic factor that was found to underly the formation of all other eye terata described above.

The following two examples may suffice:

On cross sections (fig. 80) of the microphthalmic embryo illustrated in figure 20 (p. 494) the following view is presented. One eye, while being very small, is seen to be fairly well developed, the retinal layers of the optic cup and the lens being well differentiated. The optic nerve of this eye is very clearly seen to enter the optic lobe of the opposite side. The other eye is much more defective. It consists of a rather poorly differentiated optic cup enclosing a lens. One side of the wall of this optic cup is invaginated and partly surrounds another lens. Medianwards from and near to this eye is an undifferentiated mass of apparently ophthalmic tissue with a large well differentiated lens.

The embryo then, as we see, possesses virtually three eyes, one of which in structure approaches the norm. Of the other two eyes the first is poorly differentiated and possessed of two lenses, while the second is represented by an undifferentiated optic vesicle with a lens.

The ophthalmoblastic material of both sides has suffered lesions, which resulted in the breaking up of one optic anlage into two and in the small size of the other eye due to destruction of a part of the potential eye anlage of this side. The injury sustained was a rather severe one for it affected also the brain, the bilaterality of which is obscured (as seen in fig. 80), and the rest of the body. However, it is at the anterior end of the embryo's body where most damage seems to have resulted from the process of destructive dissociation (blastolysis).

The general defects are usually even more extreme in embryos in which on examination in toto only a small rudiment of an eye, like a fragment of the pigment epithelium, is found, or where no eyes at all can be detected (cf. figs. 23 and 24). As a rule, it is found on microscopic examination of sections that most anophthalmic embryos possess poorly differentiated and deeply buried eye anlagen, sometimes with a profusion of very small lenses. In figure 49, which is a transverse section through the anterior head region of the embryo in figure 23, two optic vesicles of unequal size can be seen with lenses of corresponding sizes. The simultaneous appearance in the same section of a rudimentary ear vesicle, the unequal size and proximity to each other of the rudimentary optic cups as well as the distortion of the brain would seem to well warrant the assumption of blastolysis as the morphogenetic factor responsible for the defects of this embryo.

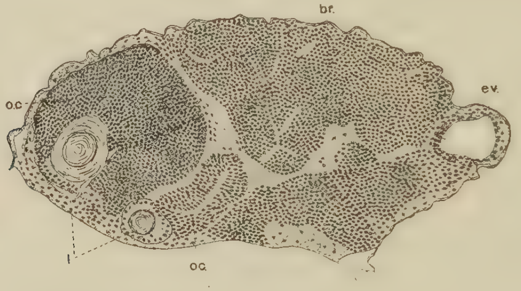

Fig. 49 Camera lucida drawing of a transverse section through the head of the embryo in figure 23. *o.c.*, optic cup; *l.*, lens; *br.*, brain; *e.v.*, ear vesicle. × 125.

C. THE MORPHOGENETIC FACTORS UNDERLYING THE ORIGIN OF EYE TERATA

1. *Stockard's inhibition theory*

The analysis of the morphogenesis of ophthalmic terata has been attempted repeatedly and with varying success. Good reviews of the opinions of the earlier writers on the subject were already given by Spemann ('04), v. Hippel ('09) and Schwalbe and Josephy ('13), and to these the reader may be referred. No distinction is made by most authors between the various degrees of the synophthalmic condition and cyclopia, i.e. the presence of a single median eye. Thus, their interpretation of the morphogenesis of cyclopia, applies to the whole so-called 'cyclencephalic' group.

Two views have been advanced to account for the morphogenesis of these monstrosities. According to the one represented by Huschke ('32), Dareste ('91) and very recently advocated by Stockard ('09, '10 a, '13) 'cyclopia' is a condition in which the separation of what they consider the originally single optic anlage has been inhibited.

Opposed to this view is the theory of fusion of two optic vesicles as underlying the formation of 'cyclopia,' which was originally advanced by Meckel ('26), and which in a modified form has recently been advocated by most investigators of the subject and particularly by Spemann.

This author (Spemann '03, '04) has with an entirely different object in view constricted eggs of Triton taeniatus by placing in the two-cell stage a ligature around the first cleavage furrow in relation to which it was somewhat oblique. As a result of this operation he obtained embryos in which the anterior end was doubled to a greater or less degree, depending upon the degree of constriction. In many of the embryos thus treated Spemann observed that one of the doubled heads (or parts of the head) thus resulting was normal while the other which, owing to the oblique ligature was narrower, had defective, usually synophthalmic and sometimes cyclopean eyes. Since the eye deformity was mostly found on the part distal from the embryo's main body axis, Spemann ('03, '04) concluded that the 'cyclopean' deformity produced by him was due to a defect because it resulted from the destruction of the area which would normally be the area between the eyes. While not inclined to support unreservedly the hypothesis originally advanced by Meckel ('26), namely that the synophthalmic or cyclopean condition results from a secondary fusion of two originally separate optic vesicles, Spemann leans very strongly ('04, pp. 440–441) to the view advanced by Fischel ('03) according to which cyclopia might result from a fusion *at a very early stage of development of two originally separate masses of cells which were to form the eyes but had fused before they began to undergo the process of differentiation* into these organs. With this latter view agree well the observations made by Stockard ('09) which I can confirm from my own experience and which were confirmed also by Lewis ('09), namely that the synophthalmic and monophthalmic deformities can be recognized as such already in the stage of the optic vesicle, in other words that a 'twin optic vesicle' or 'cyclopean optic vesicle,' if these expressions be permitted, comes off from the brain directly as such.

The experiments of W. H. Lewis ('09) have also a very important direct bearing on the subject of morphogenesis of teratophthalmia. This author employed the method of pricking the anterior end of Fundulus eggs in the embryonic shield stage. Various synophthalmic and one-eyed monsters resulted from these operations, depending on the degree and exact localization of the injury inflicted.

From these results Lewis concluded that in the cases of 'cyclopia' certain cells have been destroyed by pricking, which would normally form the area between the eys. Owing to this elimination of tissue, "the repair, taking place after the operation, consists of a closing together of the parts left behind . . . . . and rudiments are thus brought into contact that normally are quite widely separated, those of the eyes, for example." This is essentially an assumption of a fusion of early optic anlagen before their differentiation into optic vesicles, as underlying the morphogenesis of cyclopean and synophthalmic monsters.

Very recently Mall ('08) and Whitehead ('09) have advanced essentially identical views in confirmation of Lewis' ('09) conclusions.

Thus, as we see, the recent authors practically all agree upon the mode of formation of the 'cyclopean' eye. However, the fusion theory of 'cyclopia' does not altogether lack opponents, as the most ardent of whom we must now regard Stockard.

For a critique of this author's views the reader must be referred to Spemann's ('12 a and b) excellent discussion. While on the basis of evidence in my possession much could be added to the latter, I shall for the present confine myself largely to the discussion of the arguments which Stockard has most recently ('13) brought forth in defense of his views on the morphogenesis of 'cyclopia.'

Concluding from his experiments with magnesium chloride on Fundulus eggs and also from experiments with alcohol, ether, and chloroform-acetone solutions on the same material and with very similar results, Stockard ('09, '10a, p. 387) concluded that "the evidence strongly indicates that the ophthalmic abnormalities produced in these experiments are the result of an anaesthetic action during the early developmental stages."

The fallacy of this hypothesis of anaesthetic inhibition has become obvious since the work of McClendon who has recently ('12 a) shown that 'cyclopia' and other malformations of the eye can be produced by various other, non-anaesthetic substances. Thus in his most recent publication, Stockard ('13) no longer speaks of '*anaesthetic*[5] action,' but instead he considers his experimental eye terata as "the result of a *weakened*[5] development" which is brought about by the toxic solutions. These solutions, he argues, "all tend to suppress or arrest the development of the eye material in the brain." ('13, p. 271).

Stockard ('13, p. 254) believes

> that the eye anlage in the medullary plate is primarily median and single and normally separates into two almost equal growth regions, which develop in lateral directions reaching further and further out

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[5] My own italics.

until finally the optic vesicles come into contact with the ectoderm at the sides of the head.

On the basis of this hypothesis he now ('13, p. 273) offers the following explanation for the morphogenesis of synophthalmic and one-eyed monsters:

> . . . . the median eye anlage does not widen or spread laterally but is arrested in its primary condition; thus the growth centers are not sufficiently separated and only a single center exists, and even more than this, the arrest is to such an extent that the entire or normal amount of optic material does not differentiate. Hence one finds a median cyclopean eye consisting of an amount of eye material far below that normally present.

This for cyclopia. In the various degrees of synophthalmia he assumes that the 'developmental vigor' is less suppressed, less 'weakened.' Here the separation of the single anlage into two 'growth centers' is inhibited only to a certain (varying) degree and depending on the variation in the degree of inhibition various synophthalmic conditions, such as the "cyclopean eye showing distinctly its double composition," the "hour-glass eye or incomplete cyclopia," approximation of two separate eyes, etc., result.

For the genesis of lateral monophthalmia, finally, Stockard ('13) makes the following suggestion:

> The growth centers representing the two future eyes of an individual are rarely equally vigorous. . . . . It might be that at some critical point in development one of the future eye centers is affected after the growth centers had begun to localize in more or less lateral positions.

It is very difficult to understand why (in the same experiment!) in some embryos the inhibition of one of the potential eyes should begin after the division of the single anlage (monophthalmia asymmetrica), while in other embryos this single eye anlage should be inhibited before its division into two parts takes place ('cyclopia').

That the latter is primarily single and median in position in the medullary plate Stockard now regards as a fact, which he thinks he has established by experiments described in his 1913 paper. However, it seems more than probable that the method which he employed in removing fragments of tissue from the antero-median and the antero-lateral portion of the medullary plate has led to errors which Stockard apparently must have overlooked. The unavoidable inaccuracy inherent in the method of mechanically removing minute fragments is obvious. Even with such refined methods as Spemann has employed and with the experience and skill of the latter in such operations, this inaccuracy can not be entirely avoided, as Spemann himself has repeatedly pointed out. It must also be borne in mind that the whole area from which the eyes can presumably arise, is in the stage, at which Stockard performed his removal operations, relatively very small. How could any satisfactory degree of precision be attained in operations performed on this small area with 'fine scissors' (Stockard's method)? Is it not probable, that while cutting out the antero-median region of the medullary plate, Stockard has evidently removed also lateral material? Or, that, while attempting to cut away antero-lateral parts of the medullary plate he evidently removed too little, being anxious to avoid inaccuracies resulting from cuts carried too far from a presumably correct position? In the medullary plate, the region under discussion is—at least on superficial examination—morphologically homogeneous, and the mapping out of morphogenetic areas for analytic experiments is beset with well-nigh insurmountable difficulties, even if the instrument used for the operation be the finest conceivable. This can readily be seen from the results which Stockard reports to have obtained from his experiments. Of nine embryos in which 'narrow strips' were removed from the antero-median part of the medullary plate, "four . . . . failed entirely to develop eyes." Of the five other embryos only in one the eyes developed "to an extent approaching the normal" and "four . . . . individuals possessed highly defective eyes" ('13, p. 288).

Do such results warrant any conclusions at all? Are not the four cases, where defective eyes resulted from the removal of the antero-median tissue, at least as conclusive for the lateral position of two optic anlagen in the medullary plate as the four eyeless embryos for the median position of a single optic anlage?

Is it not safe to hold that in these experiments the results can not be read aright, owing to the great probability of an error introduced by the inadequate method of the experiment? It would seem obvious that, if experiments be performed on the said area at this stage of development with an instrument as crude as the one employed by Stockard, the deductions from the results are bound to be either erroneous or at least very unsafe in almost every case.

For the validity of this claim of the 'single eye anlage' and its 'median position in the medullary plate' Stockard ('13, pp. 274–276) attempts another proof. He holds that the position of the optic cross outside and below the brain would be inconceivable, if the optic anlagen should be lateral in position and other brain tissue be present between them. For, then, he concludes, the optic stalks instead of having a "median origin and connection" would be "attached to lateral regions of the brain from which the optic vesicles pushed out." According to his diagram presented in figure 8 (p. 276) ". . . . in the course of development the fibers of the optic nerve following the stalk reach the lateral position and must enter the brain and continue within its tissue in order to meet the nerve of the opposite side and form the cross or chiasma. Brain tissue would lie beneath the optic chiasma" and "this condition is never found in any normal vertebrate."

These deductions would have to be regarded as very important if they were correct. But, as will be seen from the following, they will not hold good.

At the outset it should be said that the optic vesicles do not 'push out.' Instead they are being pushed out by the primoridum of the brain. The parts of the brain anlage (and not of the eye vesicles, as Stockard suggests) most directly concerned in this process of pushing out the anlage for the eye, elongate more and more, until they have attained the form of optic stalks at the time when the differentiating eye vesicles have reached their final positions in the head. Their origin is just as much from tissue dorso-lateral as from ventro-median to the optic vesicle. It is true that this view is, as yet, not based on experimental evidence, but even lacking this important basis, it would seem far more reasonable and safer and far less arbitrary than the unwarranted claim that the 'optic anlage' is median and that the optic stalks are a part of it. Granting, however, that my view is correct—and there is at least a very high degree of probability in it—it is easy to understand that optic stalk tissue may be partly median in origin while the optic vesicles come from antero-lateral regions of the medullary plate. In this case, then, it is also evident that the position of the optic stalks and later of the optic nerves and chiasma ventral to and outside of the brain can not, by any means, be regarded as evidence of the median origin of the eye anlagen. Stockard's own diagrams (figs. 6 and 7, p. 275)[6] which are to prove that the eye anlage is primarily median in position would rather seem to support my arguments for the lateral origin of the latter, while his diagram in figure 8 (p. 276) most decidedly portrays a condition which is impossible, not, because the optic stalk tissue is (partly) of median origin, but because it is not a part of the eye vesicle.

From what has been said so far, it is obvious that Stockard's assumption of the single condition of the optic anlage and its median position in the medullary plate as a basis for the morphogenesis of synophthalmia and cyclopia is untenable.

From a study of my own abundant material in sections as well as from a careful scrutiny of the views presented by previous writers on the subject I have convinced myself that a *rational analysis of the morphogenesis of synophthalmia and synophthalmic cyclopia must be based on* Spemann's ('04, '12) and Lewis' ('09) *theory of a fusion of early* (pre-vesicular) *eye anlagen, due to a defect of intermediate tissue.* This conclusion I have reached in spite of the indisputable fact that the fusion theory is inadequate in the case of perfect cyclopia and that the nature of the defect that precedes the fusion has not yet been made quite clear.

In the following I shall attempt an analysis of the morphogenesis of teratophthalmia which is based on a recent physiological discovery of Child's as an important key to the understanding of the nature of chemical defects, and on the assumption of fusion of two originally separate early optic anlagen as underlying the formation of synophthalmic and cyclopean monstrosities.

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[6] Owing to the great importance of the subject here discussed the reader is advised to consult Stockard's ('13) diagrams, without which this discussion may perhaps not be quite intelligible.

2. The defect-theory of teratophthalmia

When Fundulus eggs are subjected to the influence of toxic solutions of a perceptibly injurious (but not lethal) concentration for a certain (not lethal) length of time, it can usually be found that among the many monstrous embryos which will develop, a certain rather large number will exhibit deformities of the eyes, while other parts of their bodies may appear not, or only very slightly, to deviate from the norm. In such embryos the eye deformities are often the most, if not the only, striking ones. Since the entire eggs were subjected to the influence of the toxic solution, it appears rather puzzling that the developmental product of the egg should show the effect of the treatment only at such a locally restricted area. In experiments in which mechanical methods (such as pricking—Lewis '09, or constriction—Spemann '04) are employed the local deformation can readily be accounted for by the locally restricted lesion which has been caused mechanically. In the chemical experiment, however, the treatment is not restricted to a part of the egg, and yet the effect is so often a restricted one. How can this be accounted for?

There is, so far as I am aware, only one known fact, which will account for this interesting phenomenon. This is Child's important discovery of the high susceptibility of the animal pole to noxious influences.

This author (Child '11, '12, '13, '14) has shown that if a ciliate infusorium or a planarian be subjected to the influence of lethal solutions of certain toxic substances the disintegration resulting in the death of the animal will proceed gradually from the anterior towards the posterior end of the body. The same results were obtained also in other adult invertebrates. In this manner a definite gradient of susceptibility was demonstrated to exist along the chief body axis.

Of quite particular interest in connection with my own results reported in this paper are Child's ('15 b) experiments on starfish eggs. If these were subjected at early stages of development to the influence of solutions of potassium cyanide not strong enough to kill the eggs, the resulting larvae exhibited the detrimental effect of the sojourn in the toxic solution mainly in the apical region.[7] Here, as well as in my experiments on Fundulus the part of the egg destroyed by chemical action is the one which corresponds potentially to the embryo's apical (animal) pole.

Very recently Child ('15 a) has concluded that this primary gradient from the 'animal pole' to the 'vegetative pole' is very general in organic life and that its demonstraton is only difficult in higher animals, where, owing to complex organization, the results of the 'resistance method,' by which they can be demonstrated in invertebrates, are obscured.

In the early embryo, however, before the differentiation of organs, when the physiological conditions are yet relatively simple, the assumption of such an 'axial gradient' in the susceptibility of the fish egg would seem to be justified.

If, however, this gradient of metabolic reactions exists in the fish egg, then there exists also a point of highest susceptibility and accordingly of least resistance; and this point (the animal pole) is the potential anterior end of the embryo's body. Granting this, however, it is no longer difficult to understand why the effect of the toxic solution on Fundulus eggs should, as it so often does, manifest itself in defects of organs of the anterior end of the body, and most particularly the eyes, the mouth, the olfactory pits, and the forebrain, while the rest of the body may suffer very little from the sojourn in the solution.

Many observations point to the conclusion that this injury at the apical pole which results in terata of the eyes, is caused mainly by a process of disintegration and dissociation which I have termed blastolysis. It is not easy to understand just what chemical reactions may underlie this process. They obviously depend entirely on the chemical nature of the solution employed. Thus it would seem reasonable to expect that for instance they are quite different in butyric acid solutions from those of magnesium chloride solutions employed by Stockard ('09) or alkaloid solutions employed by McClendon ('12 b). The action of some solutions may dissolve, while that of other solutions may coagulate or precipitate certain substances of the egg. However, no matter what this action may be, it certainly results in a chemical alteration, which will be the more intense, the higher the concentration of the solution or the longer the time of exposure. Accordingly, if the action be a slight or moderate one, the chemical alteration may result only in an inhibition of certain groups of cells possessing a high degree of susceptibility, i.e. these cells may continue dividing and differentiating up to a certain point, beyond which, owing to exhaustion of their chemical capacity, they are unable to proceed. Or the action may be strong enough to cause, by chemical alteration, a check of the most important physiological processes (cell metabolism and cell division) of these embryonic cells, which would result in their disintegration. No matter what chemical solution be employed, if it only is injurious to life, it will in this way, have a destructive effect. If non-lethal concentrations and lengths of exposure be employed, this destructive process will largely be restricted to the animal pole of the egg, i.e., to that part of the egg which in normal development would correspond to a certain area at the anterior end of the potential embryo's body. Since, according to the rule of the 'axial gradient' this destructive process—chemical blastolysis—begins at the animal pole of the egg and cannot proceed further, owing to insufficient strength of the solution or to timely transfer to a normal environment (pure sea-water), its effect is eventually noted in deformities at the embryo's anterior end of the body.

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[7] Very recently Painter ('15) observed that in Ascaris eggs which have by an accident come under the influence of carbon dioxide "roughly 33 per cent of the embryos (in 54 cases out of 165 examined for the point)" have sustained severe injuries at the anterior end.

According to what has been said so far the sequence of events leading to the deformities of the eye is, then, more or less the following.

A small part of the egg in the earliest stages of development corresponding to a restricted area at the anterior end of the future embryo's body sustains a chemical lesion, i.e., it becomes so altered chemically as to be incapable of the reactions necessary for its further normal development. The part of the egg thus incapacitated, is potentially the region anterior to and between the future optic anlagen or even the region of those anlagen. This affected area goes on developing up to a certain point beyond which, owing to the exhaustion of its chemical capacities, it loses its correlation with the whole, i.e., with the rest of the embryo-forming material and becomes eliminated by dissociation. Or, the affected area may, at that critical point of chemical incapacity, be permanently arrested and retain the characteristics of this early stage of development (some cases of anophthalmia), while the rest of the embryo may develop and differentiate further.

The size of this restricted area of blastolytic lesion at the anterior end of the potential embryo's body axis is probably subject to considerable variation. Thus it may comprise the mass of cells which would normally correspond to the future interocular area and cause an approximation of the potential optic anlagen or, it may extend over the latter ones and eliminate parts of them, while the uninjured parts would fuse after an approximation resulting from the healing of the wound, and form any one of the various degrees of the synophthalmic condition. Again, the injured region may comprise parts of the ophthalmoblastic material of both sides and very little of the potential interocular area. The remnants of the optic anlagen may develop and differentiate fully into eyes of strikingly small size of the microphthalmic monsters. Or, the lesion may comprise the whole of one optic anlage and little or no material of the future interocular area. In that case the embryo will develop into a perfectly cyclopean embryo, if, owing to subsequent regulation, the uninjured potential optic anlage is shifted medianwards, or into an asymmetrically monophthalmic monster, if no such change in the position of the uninjured or less injured ophthalmoblastic material takes place. Wherever in such cases the sustained injury is of a still higher degree, i.e., if only a fragment of one optic anlage survives, the cyclopean or asymmetrically monophthalmic eye, formed from it, will be of a correspondingly small size. And, finally, if the entire ophthalmoblastic material is destroyed by the blastolytic process, the defect will result in anophthalmia vera.

At this point it should be noted that there is yet another kind of anophthalmia, which is not due to blastolytic destruction but apparently to an inhibition. In anophthalmic embryos of the latter category there are always found on microscopic examination eyes which owing to the exhaustion of their chemomorphic capacity, have remained at a very early stage of development (figs. 23 and 24, p. 494 and fig. 49, p. 520). This, however, is, I believe, the only instance where an ophthalmic deformity is due to inhibition, while all synophthalmic and one-eyed conditions owe their origin to a defect brought about by blastolytic elimination.

For a better understanding of the morphogenesis of ophthalmic terata it is also necessary to consider the time at which blastolysis brings about the changes leading to their formation. Since it can be shown that in Fundulus eggs under the influence of toxic solutions the chemical injury follows the rule of the 'axial gradient,' i.e., it proceeds from the anterior end posteriorwards, it seems safe to assume that the blastolytic lesion is sustained at a very early, primitive stage of development, long before differentiation of organs has yet begun.

In a previous paper (Werber '15 c, p. 558) I have advanced the view that the blastolytic injury is sustained before the formation of the embryonic shield. To be more precise, it may be said that this elimination of destroyed material most likely occurs at a late 'Randwulst'-stage of the germ-ring. For it is at this stage that very important events take place which eventually lead to the organization of the embryonic body and differentiation of tissues and organs. At that time of transformation of the blastoderm into the embryo which is accompanied by rather active movements or shiftings of the embryo-forming material, the contact between the sound, unaltered part of the latter and the chemically destroyed one is loosened more and more until it is lost entirely and the altered fragment breaks off.

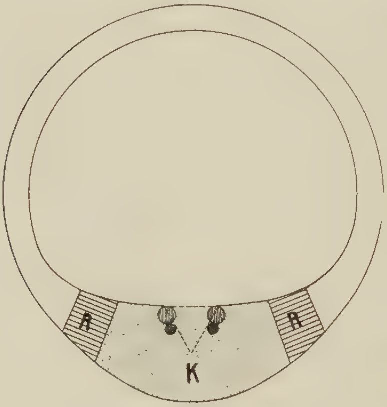
This will at once become apparent if we recall the facts regarding the formation of the embryo in teleosts so well established by the investigations of Kopsch ('96, '04). According to this author the formation of the embryo comes about in the following manner ('96, p. 120):

> An dem zelligen Randring (der Keimscheibe 24 Stunden nach der Bildung des ersten Umschlages) muss man zwei Bezirke unterscheiden. Einen embryobildenden und einen nicht (direct) embryobildenden. An dem embryobildenden Bezirk, welcher an der Stelle der ersten Einstülpung gelegen ist, haben wir weiter zu unterscheiden einen der Medianlinie näher gelegenen Teil, dessen Zellen den Kopf des Embryos bilden, und jederseits lateral von diesem Bezirke Zellengruppen, welche im Laufe der Entwickelung in der Medianlinie zusammenkommen, und den Knopf bilden. Der Knopf stellt ein Wachstumcentrum vor, von welchem Rumpf und Schwanz gebildet werden, wobei Zellen des nicht direct zum Aufbau des Embryos verwendeten Teiles des Randringes im Laufe der Umwachsung des Dotters zum Knopf gelangen und dort ebenfalls zur Bildung des Embryos benutzt werden.

In both the area which is to form the head (K) and in the parts which are to form the 'Knopf' (figs. 50 and 51, p. 534) the organs of the head and trunk are potentially contained. For experiments of Kopsch have shown that if parts of any one of these areas be destroyed the organs which these eliminated fragments potentially represent, will be lacking (Kopsch '96, p. 121).

Fully in accord with these data is the view which I have expressed above, namely that terata of the head owe their origin to blastolytic injury at the anterior part of the area which is to form the head. This area would correspond to the area K in Kopsch's diagram and the blastolytic elimination of an anteromedian chemically incapacitated fragment of this area must be assumed as taking place before the 'Knopf' has yet been formed, or thereabout. The eliminated fragment would contain parts of the material potentially representing maxillary and mandibular arches, the olfactory pits, the tissues of the interocular area and very often more or less of the ophthalmoblastic material. Following a suggestion made by Schwalbe and Josephy ('13, p. 205–206) I have attempted to portray this elimination diagrammatically, employing Kopsch's diagram as a basis (fig. 50).

In the dotted area K, which is the primordium of the head, the two horizontally barred circles represent groups of cells which would normally develop into the mouth and olfactory pits, while the two cross-barred circles immediately below represent the ophthalmoblastic material of both sides. If it now be imagined that blastolysis has eliminated a wedge of tissue which as seen in the diagram, is directed with its point posteriorly, while it is broadest anteriorly, this wedge would contain, more or less, the potential interocular area, parts of the potential mouth and the potential olfactory pits of both sides. A considerable part of the potential forebrain would in this way also be eliminated. The gap thus resulting will soon close, owing to a contraction brought about by the elongation which accompanies the transformation of the head primordium of this stage into the head. What would be the final result of the elimination of such a wedge of tissue and the subsequent closure of the wound? A glance at figure 50 would show that the potential



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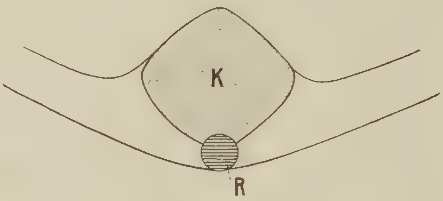

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Fig. 50 Diagram of germ-ring with thickened embryo-formating area ('Keimrandwulst')—modified from Kopsch.

Fig. 51 Diagram of the early steps in the formation of the embryo from the thickened area of the germ-ring (from Kopsch).

anlagen of the mouth, olfactory pits and the eyes of both sides would in this way be brought into more or less close approximation or even into contact. Various degrees of the synophthalmic deformity would thus eventually result from the more or less complete union of both ophthalmoblastic areas. Incidentally the elimination of parts of the area potentially representing the mouth and olfactory pits will by fusion of their remnants lead to the formation of an elongated, more or less pointed mouth—the 'proboscis'—and to fused olfactory pits.

A few words would now seem necessary regarding the size of the eliminated fragment and the distances between the respective organoblastic areas of both sides. While in our diagram, by which only a very rough portrayal of the presumable conditions is attempted, the eliminated area is relatively large and the respective organoblastic areas are pretty far apart, it is not at all meant to convey the idea that they are so in reality. Concluding from what appears to be lacking in the developed embryo and considering the fact that growth is an increase in volume, this destroyed anterior part of the head primordium must be conceived of as being relatively very small in size. It follows from these considerations that the bilateral ophthalmoblastic areas are at this stage relatively much nearer each other than they would be at a somewhat later stage, e.g., in the embryonic shield. Granting this, however, it is easy to account for the fact that the cyclopean eye is often much smaller in size than the normal eye. For, at this stage the eliminated area may often contain besides the potential interocular area a whole ophthalmoblastic area of one side and a part of the same area of the other side. The remnant of ophthalmoblastic material may develop laterally (monophthalmia asymmetrica) or into the median, genetically single eye of perfect cyclopia, if, owing to subsequent regulation it has come to occupy a median position. In the case of a small cyclopean, genetically synophthalmic (i.e., 'fused') eye it might be imagined that the destroyed 'wedge' of tissue contained a great deal of both ophthalmoblastic areas and that the remnants have, after fusion, developed into a median eye smaller in size than the normal eye. However, although I do not deny the possibility of such small size in synophthalmic cyclopia, I would not leave unmentioned the fact that in my observations made on a great number of sectioned teratophthalmic embryos I have not yet had occasion to record a single such case. Examination of the figures in Stockard's ('09, '10 a) papers also very forcibly suggests that all cases of small cyclopean eyes which he pictures, represent perfect cyclopia, i.e. a condition of median, genetically single, one-eyedness, where a fragment of ophthalmoblastic material of one side developed into a small, but whole, eye in the median position, which it secondarily has come to occupy.

On the basis of our conception of the morphogenesis of ophthalmic terata, namely that the latter are due to a defect it is easy to account for the unquestionable fact that neither the perfectly cyclopean nor the synophthalmic eyes (cyclopia synophthalmica, synophthalmia unilentica et bilentica) are double the size of one normal eye, as Stockard ('13, p. 270) postulates they would have to be, if the fusion theory be regarded as correct. Such eyes simply cannot be "equal in mass to the two normal eyes fused" because the fusion in the synophthalmic eyes is due to destruction of a part of the eye forming material. This, however, meets an important and justified objection which Stockard has raised against the fusion theory of 'cyclopia.'

It is, no doubt, true that, while snyophthalmic deformities can be accounted for on the basis of this fusion theory, it would fail if extended to other ophthalmic terata. For, perfect cyclopia cannot, as was pointed out above, be regarded as due to fusion. Nor can it be denied that, as Stockard ('13, p. 278) remarks, the genesis of asymmetric monophthalmia and microphthalmia have yet to be accounted for. But while we admit these limitations of the fusion theory, it must be borne in mind that its most fundamental element—the exceedingly suggestive defect hypothesis of Lewis and Spemann will alone account for asymmetric monophthalmia and microphthalmia as well, as for perfect cyclopia.

The defect which in synophthalmia we have assumed to consist in a blastolytic elimination of a fragment of tissue from the antero-median portion of the head primordium may, and, no doubt does, vary in quantity and location. The eliminated 'wedge' may involve an antero-lateral fragment only, which contains the ophthalmoblastic material of one side and in this case the embryo will lack one eye (asymmetric monophthalmia). Or, the 'wedge' may be a very broad and short one and involve parts of the ophthalmoblastic areas of both sides as well as a small antero-median portion. The result will be an embryo with small eyes, as a rule nearer each other than are normal eyes, and with deformities of the mouth (often proboscis-shaped) and the olfactory pits (microphthalmia).

Neither the unwarranted assumption of a single median eye anlage and an inhibition of its 'developmental vigor' (Stockard) nor, much less yet, degeneration due to deficiencies in the circulatory system as Loeb ('15) assumes, can be expected to solve the difficult problem of teratophthalmia. For, these deformities are clearly due to a defect of the undifferentiated embryonic primordium. With the possible exception of such cases of anophthalmia (in which on sectioning deeply buried rudimentary eyes are found) where an inhibition cannot be excluded, all eye malformations can be accounted for by a blastolytic defect. And the localization of the latter can, in turn, be understood, when we assume the presence of an antero-posterior axial susceptibility gradient, which Child's investigations have made so highly probable.

D. DEFORMITIES OF THE BRAIN

In nearly all pathological embryos recorded in our observations the central nervous system is more or less deformed. No detailed study of the malformations of this system has yet been made, and the few observations which will be recorded at this place pertain largely to the brain in teratophthalmic embryos.

In our description of ophthalmic deformities same malformations of the brain have already been pointed out (cf. pp. 503, 506, 508, 509, 511, 512, 513, 515 and 520). It was shown that in synophthalmic and cyclopean monsters the forebrain is single, there being no division into a right and left lobe. This is evidenced very plainly by figure 52, which is a transverse section of a synophthalmic embryo, as well as by the cross sections of the cyclopean embryos illustrated in figures 63, 66 and 70. The mid-brain and hind-brain are mostly bilobed and otherwise symmetric. Sometimes, however, and usually only in cases of perfect cyclopia, the single unilobed condition may be found to obtain also in the mid-brain (cf. fig. 48, p. 513, also figs. 65 and 67) and even in the hind-brain (fig. 71).

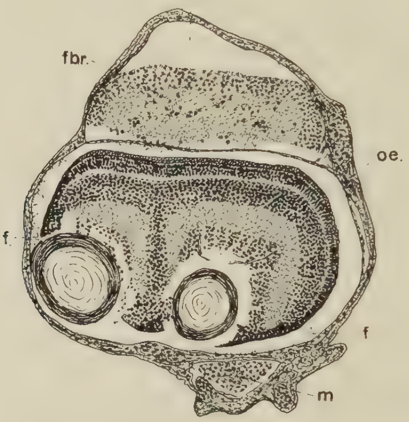


Fig. 52 Camera lucida drawing of a transverse section through the eye region of a synophthalmic embryo from acetone solution (35 cc. gram molec. to 50 cc. sea-water) thirty-two days old. *fbr.*, fore-brain; *oe.*, oedema; *e.oe.*, extracerebral oedema; *f.*, fibrin; *m*, mandible. × 100.

An important clue to the genesis of this disturbance in the symmetry of the forebrain (and rarely also of the mid-brain) is given by the size of the latter. In transverse sections it is often much smaller in area than the synophthalmic or cyclopean eye, as a glance at text-figures 48 and 52 and figures 63, 65, 66, 67 and 70 will convince. This indicates clearly that something is lacking in such brains. In other words, we are here dealing with defects. They are undoubtedly the same defects which resulted in the deformities of the eyes of these embryos, and are due to blastolytic elimination of a 'wedge' of tissue from the anterior or anteromedian portion of the early, undifferentiated head primordium. In most embryos which exhibit no other than eye deformities, this destructive blastolysis affects the forebrain only, while occasionally the mid-brain, or a part of it, may to some extent be found to be involved in the process of destruction. Among many embryos which, while being teratophthalmic, appeared to be 'normal' in other respects, I have found one in which blastolytic fragmentation in the region of the mid-brain (one optic lobe) is very apparent. A transverse section at the level of the mid-brain of the embryo (fig. 13), whose one eye only was defective, the other being normal, is shown in figure 74. Examination of this figure shows that the left optic lobe (on the right side of the figure) is fragmented, the greater part of it being delaminated in such a manner that a wide furrow resulted between the two fragments of the lobe. The break through the optic lobe is, however, not complete, for if more posterior sections be examined, it can be seen that, at the basal (ventral) part, the lobe is unbroken. This finding, I think, is highly indicative of the blastolytic nature of the process which brings about the defects of the brain.

Our observations on the brains of teratophthalmic Fundulus embryos agree well with those made on the brains of human ophthalmic monsters. Here, too, the mid-brain and hind-brain usually (in cases where cyclopia is the only deformity present—Ernst '09) are normal while the forebrain is small and unilobed. The single condition of the latter is so well known as to have even led some authors to the assumption that it is responsible for cyclopia. In our opinion it is syngenetic with the deformities of the eyes, the olfactory organ and the mouth, i.e., all these deformities are genetically due to the same defect—the blastolytic destruction of a certain (more or less wedge-shaped) part at the anterior end of the early head anlage.

The chemical alteration which underlies the blastolytic lesion is, as has been pointed out repeatedly in the preceding, the greatest most anteriorly and diminishes posteriorwards along a line corresponding with the future embryo's chief body axis. Most anteriorly this alteration leads to destruction and elimination, while decreasing posteriorwards, it becomes gradually less and less destructive and only so interferes with the typical physiological reactions, as to diminish the capacity for normal differentiation. Thus in more posterior regions of the head of some teratophthalmic embryos the result is not an elimination of tissue but often a developmental inhibition.

Such an inhibition manifests itself mainly in the cells of posterior parts of the brain. They sometimes remain in the neuroblast stage in spite of the relatively advanced age of the embryo. This can be observed particularly in embryos in which the malformation has involved more than the eyes only.

Another observation which points to inhibition is that concerning the presence, usually in the mid-brain and more posterior parts of the brain, of many large tissue spaces (fig. 69), which are sometimes clear and empty while often they may be filled with fibrin (figs. 45, 46 and 48). These spaces evidently represent persistent early vascular anlagen which, owing to chemical incapacity, have been unable to develop any further and connect up into continuous vascular channels. The probability of the latter assumption is much enhanced by the fact that the heads of such embryos during life, owing to accumulation of fluid in the tissue spaces, are sometimes greatly distended.

This intracerebral oedema in the fish embryo, resulting apparently from the absence of a continuous system of haemal and lymphatic drainage, is, since the fish brain has neither lateral ventricles nor a choroid plexus, in a manner comparable to the congenital internal hydrocephalus of man. Such a condition of hydrops is often found to exist also in the cranial cavity which in these cases is unusually distended and sometimes contains fibrin (cf. figs. 46, 48 and 52). The extracerebral oedema is also probably due to blood—or lymph vascular imperfections resulting from a developmental arrest. It is comparable to the human congenital external hydrocephalus, i.e., a condition, where an accumulation of fluid is found in the arachnoidal space. Both the extracerebral and the intracerebral oedema may sometimes be found in the same embryo (figs. 46 and 48).

The suggestion would thus seem to be at hand that both forms of hydrocephalus in man may be due to local inhibition of the development of the blood and lymph vessels in the head region and the resulting lack of adequate drainage. Here, however, much further study is necessary. Careful anatomical investigations of well diagnosed cases of both forms of congenital hydrocephalus in man may possibly bear out the validity of our assumption. Much might be gained also from studies of the blood and lymph vessels of oedematous regions in experimentally deformed Fundulus embryos.

E. THE MICROSCOPIC ANALYSIS OF SOME TERATOMATA (THE 'SOLITARY EYE' AND THE 'ISOLATED EYE')

The tendency of Fundulus eggs when treated with butyric acid and acetone solutions, to give rise to partial embryos (meroplasts) has been noted in a preceding chapter (p. 499). It is obvious that a careful anatomical study of these monsters is very desirable, for their internal organization often discloses conditions of great ontomechanical significance. At the present time, while this study is yet incomplete, it may suffice to state that from microscopic examination of such embryos one gains the impression that the entire embryo-forming material has suffered greatly from blastolysis and that after a haphazard reconstitution of the surviving, but disarranged, parts of the germ the latter have developed into an embryo which is neither a whole, nor a symmetrical part of a whole. True anterior hemiembryos, i.e. formations in which an anterior half of the body (e.g. beyond the level of the pectoral fins) is present, while the rest of the body is lacking, have also been found. However, such cases are relatively rare. For, usually the blastolytic injury, whenever it is of a high degree, affects, to a greater or less extent, the whole embryo-forming substance.

Thus, it may happen that nearly all of the embryo-forming material suffers destruction and that only a very small fragment of it survives, which, however, is capable of further—independent—development and differentiation.

As the most interesting cases of this category may be regarded some eggs in which all that can be found on the yolk-sac (excepting some rudimentary bloodvessels) is a fragment of tissue with an eye (figs. 38–41) which may be single or composite (synophthalmia, fig. 39). In the fragment of nervous tissue from which such an eye arises the form relations of a brain do not exist, nor is it possible to recognize what parts of the brain are represented by the fragment.

One of such eggs with a 'solitary eye' has been briefly described in a previous paper (Werber '15 c). The significance of such formations being obvious, it seems desirable to give at this time a fuller description of it as well, as of two more eggs with solitary eyes which have been studied in sections.

In figure 40 is seen an egg without an embryo but with several tissue fragments on the yolk, distant from each other, one of which has given rise to a small eye discernible in toto.

The sections into which this egg has been cut, run parallel with the vertical axis of the solitary eye. Examination of the entire series (only one section missing) discloses the following conditions.

Nothing can be found in the yolk in any of the sections. Thus the possibility is entirely eliminated that the embryo had sunken into the yolk leaving one of its eyes (constricted off) on the surface. Several blood lacunae can be seen on the yolk-sac, some filled with erythrocytes, while others are densely packed with large cells of the appearance of leucocytes. The sections through the tissue fragment from which the eye arose, present a view somewhat similar to a transverse section of a rudimentary spinal cord (fig. 53). In further sections, proximal to the eye, this fragment of nervous tissue increases in volume so much (fig. 54) that it seems safe to regard it as a—malformed, highly defective—brain. Partially in contact with the latter is the lens, which appears in further sections, after part of the optic cup has already come into view. The whole eye is very small and is rather deeply imbedded in the yolk (fig. 55). Of its component parts the retina, and the lens are best developed. No iris is present, and the cells of the cornea are poorly differentiated. The space relations of the eye to the 'brain' which has given rise to it are much distorted, the brain enclosing part of the eye.

Of the other tissue fragments, which have been observed in the living egg, as well as after fixation, one has proved to be another solitary eye. This other eye is also seen to arise from nervous tissue, which, obviously, is a (very defective) brain (fig. 56). The retina of this second solitary eye is in a very rudimentary stage of differentiation, no pigment layer is present, nor an iris; the cornea is very poorly differentiated, while the lens is practically perfect. This second eye is much smaller than the first one described, from which it is 262μ distant.

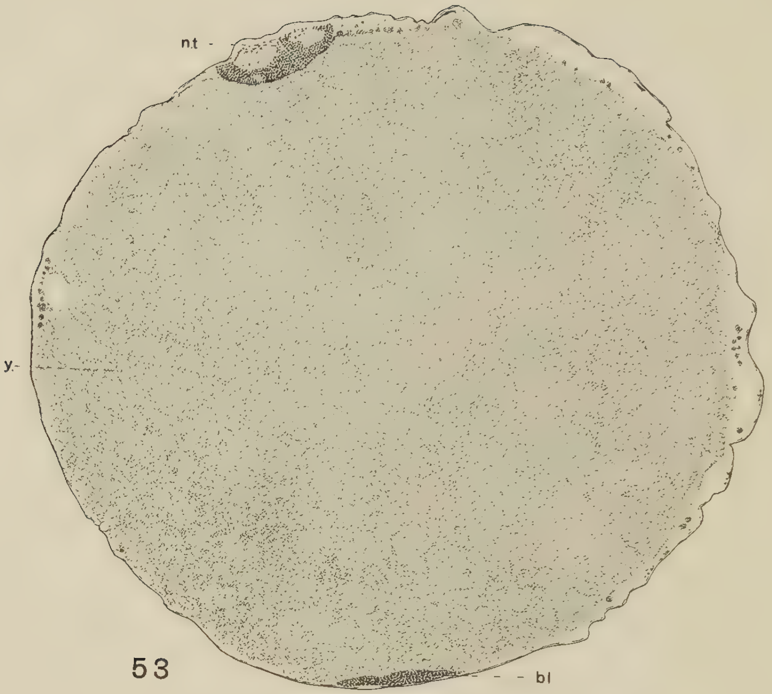
These microscopic findings are very significant for the analysis of the morphogenesis of the solitary eyes of this egg. Since no embryo can be found in the egg, it is obvious that with the exception of very small fragments all of the earliest embryonic primordium has suffered destruction. It is likewise obvious from the great distance between the surviving fragments which have given rise to solitary eyes that they have secondarily been shifted to distant parts of the yolk. The destruction of the embryonic anlage is possibly in the nature of a blastolytic disintegration (chemical blastolysis), while the apparent dispersion of its small surviving remnants would seem to be due to an increase in the eggs internal osmotic (exosmotic) pressure (osmotic blastolysis).

That both chemical (toxic) and osmotic blastolysis underlie the origin of such meroplastic formations is indicated by the next case of 'solitary eye' now to be described.

On examination in toto (fig. 40) of the transparent egg (both while living and after preservation) no embryo could be seen anywhere. All that could be found on the yolk was a small meroplast, which made the impression of a very large eye with a protruding, unproportionally large lens.

The true conditions obtaining in this meroplast, however, are somewhat different. For, examination of sections proves unmistakably that this meroplastic formation presents a case of solitary synophthalmia.

In the first three sections (fig. 57) there appears the lens of one of the eye components (left). It is perfectly well developed and surrounded by an epithelial capsule. On one side of the cross-sectioned lens is seen a thickened mass of tissue which



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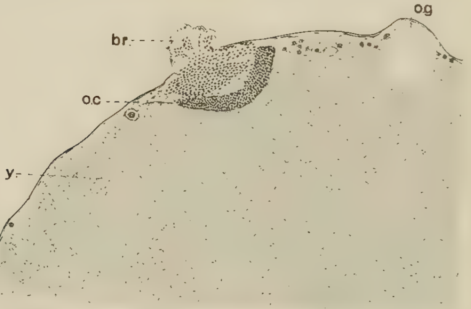

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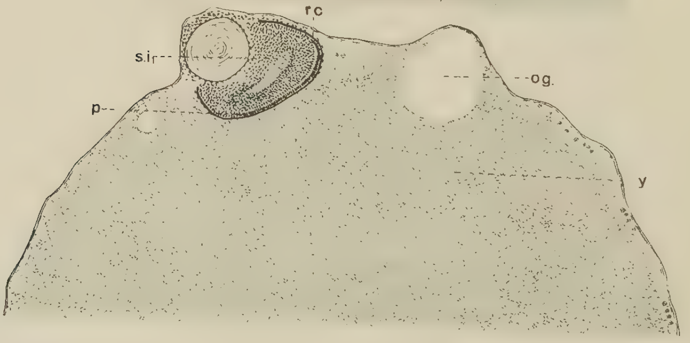

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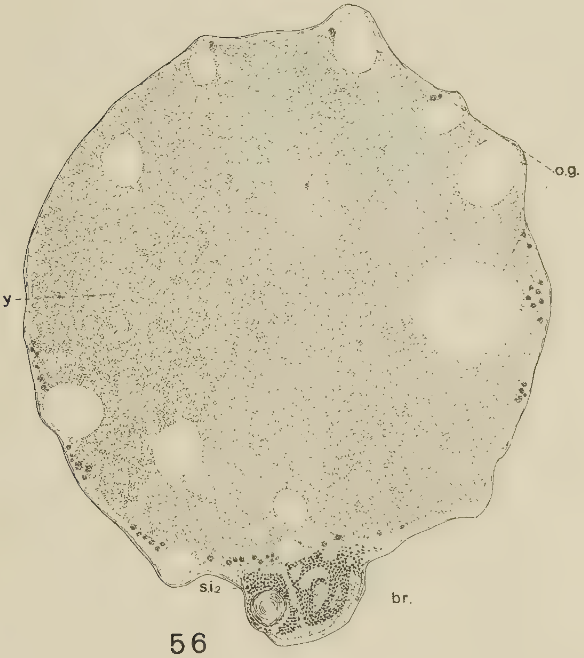

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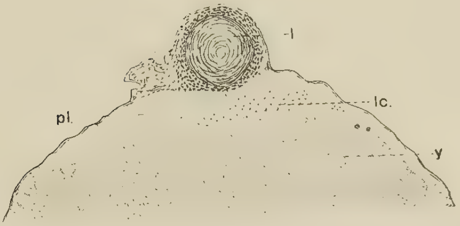

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Figs. 53 and 54 Camera lucida drawings of sections through egg in figure 40, *n.t.*, nervous tissue fragment, (brain, *br.*), from which solitary eye arose. *o.c.*, optic cup; *b.l.*, blood lacunae; *y.*, yolk; *o.g.*, oil globule. × 78.

Figs. 55 and 56 Camera lucida drawings of sections posterior to those in figures 53 and 54. $s.e_1$., first eye-teratoma ('solitary eye'); *r.c.*, rods and cones; *p.*, pigment epithelium; $s.i_2$ second 'solitary eye;' *br.*, brain; *y.*, yolk; *o.g.*, oil globules. × 78.

Figs. 57 and 58 Camera lucida drawings of sections through egg with eye-teratoma ('solitary synophthalmia') in figure 39, *l.*, lens; *l.c.*, lymphocytes; *pl.*, plasma; *br.*, brain fragments; *p.*, pigment epithelium; *n.*, optic nerve; *y.*, yolk; *o.g.*, oil globule. × 78.

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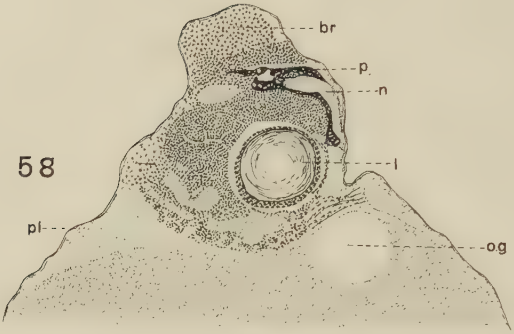

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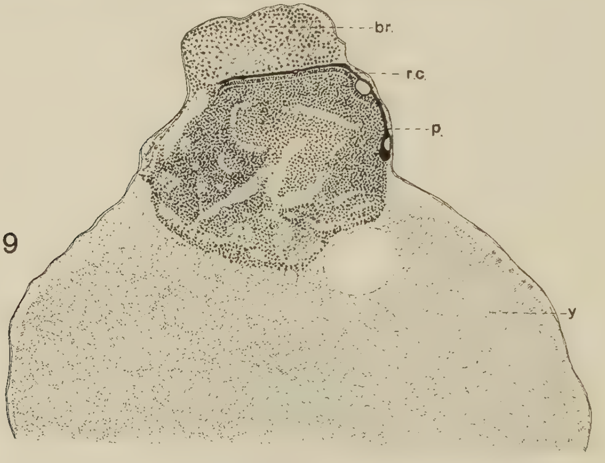

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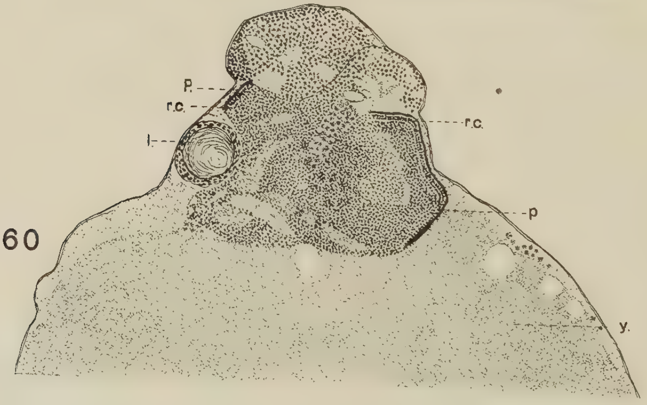

Figs. 59 and 60 Camera lucida drawings of sections posterior to those in figures 57 and 58. *br.*, brain; *r.c.*, rods and cones; *p.* pigment epithelium, *l.*, lens. × 78.

makes the impression of an ill-differentiated cornea. Between the lens and the yolk there is to be seen a very wide space filled with a plasma-like mass and lymphocytes. In farther sections an optic cup and still further a small unilobed, oedematous brain comes into view (fig. 58). A part of the pigment epithelium is also seen in this section; it partly surrounds a nerve trunk (apparently the optic nerve?), whose course is a very tortuous one. In further sections posterior to the lens the rods-and-cones-layer of the retina can be easily recognized (fig. 59), while the other layers seem to be ill-differentiated and (owing to blastolysis) considerably intermingled.

In still further sections through the meroplast the lens and the optic cup of the second eye component gradually come into view (fig. 60). The optic cups of both eye components are fused for the most part and if the whole series of sections be followed out, only the bottoms of the optic cups appear to be separate. The analogy between the morphology of this case of solitary synophthalmia and that of bilentic synophthalmia described in a preceding chapter of this paper (cf. p. 503) is evident.

What is the morphogenesis of this meroplast? The fusion of two early undifferentiated eye anlagen has evidently occurred after chemical blastolysis has eliminated a wedge of intermediate tissue. This blastolytic process has probably taken place while the egg was in the toxic solution, while on subsequent transfer to pure sea-water owing to increased imbibition of sea-water, resulting from the treatment of the eggs with acids, osmotic pressure has apparently destroyed all of the embryonic primordium excepting the small part which has given rise to the synophthalmic head fragment.

One more egg with a solitary eye may yet be briefly described. In figure 38 which illustrates it, there can be seen a rather large, tissue fragment with a very large eye. Close to it is a large oedematous (pericardial?) vesicle. Some small blood vessels have also developed, but there is neither a heart nor a continuous system of circulation. Owing to the considerable size of the tissue fragment from which the eye arises, as well as to its peculiar shape, the interpretation of the case in toto appeared difficult.

In sagittal sections, however, it was recognized that the large size of the meroplast was due to oedema. In figure 81 is presented such a section which is near the longitudinal midline of the meroplast. The view is that of a transverse section of a rather distorted diencephalon and a large, longitudinally sectioned eye. The latter is well developed; the pigment layer is rather delicate, the rods-and-cones-layer of the retina, which is considerably distorted, is well differentiated, as is also the lens. Between the pigment epithelium and the other retinal layers of a large part of the eye there is to be seen an oedematous space filled with plasma and lymphocytes. This oedematous condition is responsible for the unusually large size of the eye, as well as for the distortion of the retina. For, in further sections, where the oedematous space gradually disappears, the wall of the optic cup has a practically normal appearance. Both the eye and the brain are situated between the yolk-sac and the yolk. The brain, while very close to the eye, is partitioned off from the latter by an ectodermal lamella. It thus appears to be almost enclosed in a separate cavity which is surrounded by the blastoderm on all sides but one. This cavity is, however, not entirely taken up by the brain, for as seen in figure 81, it contains several large empty spaces filled with either plasma or leucocytes. These findings explain the large size of the meroplast as well as the peculiar shape of the tissue fragment from which the eye has arisen.

The three cases of solitary eye described above furnish ample evidence for the fact that a fragment of the medullary plate may be capable of independent development to such a high degree as to give rise to a well differentiated eye.

A somewhat similar case has been recently reported by J. Loeb ('15, p. 96). In the monstrosity which he produced from a Fundulus egg by low temperature "one eye with a lens and the tail was all that could be recognized" (cf. Loeb's fig. 13). His interpretation is to the effect that "the whole embryo had developed but the eye had survived while the other parts perished."

Something, no doubt, did perish in the egg which Loeb describes but it did so before the formation of the embryo. This early death of the part of the germ which was to form the part lacking in the described egg is due to disintegration caused by the noxiously low temperature. The parts of the germ which have survived have eventually developed—independently—into a head fragment with an eye, a clubby tail and a heart. The analogy of this case with the 'solitary eye'-formations recorded in my work is obvious.

That such cases are not due, as Loeb suggests, to the death of a part of the already formed embryo is further evidenced very strongly by some eggs in which besides a complete, but teratophthalmic embryo, there has developed independently and at a great distance from the latter a small tissue fragment with an eye. In the course of the experiments reported in this paper three such eggs with "isolated eyes" have been recorded[8] (cf. figs. 29, 42 and 43).

One of these eggs (presented in figure 42) may now be briefly described. The embryo is asymmetrically monophthalmic and is also seen to lack all fins. No other defects could be observed in toto. On the yolk-sac very near the distal end of the unusually large pericardial vesicle a small round tissue fragment can be seen. Below it and at a very great distance from the embryo a much larger tissue fragment with a well developed eye can be observed.

On microscopic examination of sections of the entire egg it could be recognized that the smaller tissue fragment is a rudimentary eye and that the interpretation of the larger tissue fragment as an isolated eye is perfectly correct.

The egg was cut into sections running transversely through the embryo. In the most anterior sections through the embryo (fig. 82) there can be seen on one side of the yolk the malformed brain of the embryo with the anterior part of the eye, while on the yolk's opposite side the smaller one of the isolated fragments comes into view. This tissue fragment can, on careful examination, be recognized as a very rudimentary optic cup with an imperfectly differentiated lens. The latter is (as can be made out without difficulty even at the low magnification of the figure) in a stage transitional between the cellular of the lens-bud and fibrillar of the fully developed lens. In sections more posteriorly, and beyond the level of this first isolated eye, the second and larger one comes into view exactly opposite the monophthalmic embryo's brain (fig. 83). Two small lenses are first seen in this eye, the retina of which has an early embryonic, rudimentary appearance. In more posterior sections, the smaller of the two lenses disappears, while the larger one appears to be of a size to fit the eye, in which at this level the retina appears to be better differentiated, its rods-and-cones layer being clearly discernible (fig. 84). In still further sections posterior to this larger isolated eye there comes into view the highly malformed brain fragment from which the eye has arisen.

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[8] More recent experiments (not yet published) have yielded a relatively great number of eye-teratomata of both kinds (with and without an embryo).

There is no connection whatsoever between these two isolated eyes and the embryo. Like conditions obtain in the two other eggs with isolated eyes (figs. 29 and 43) which have also been examined in sections.

No degeneration, as suggested by Loeb, can account for such cases. For, there are no processes of degeneration to which the already formed embryo might be subject other than (occasionally) necrotic (cytolytic?) changes. The latter, however, lead to the entire embryo's rapid death.

These teratomata present one more striking proof for the correctness of the assumption that the injuries sustained by the eggs, due to a noxious alteration of the environment, are of a blastolytic nature. The 'isolated eye' as well as the 'solitary eye' develops from a fragment of the anterior end of the potential embryo's body. In the first case the remainder of the germ's substance survives and develops into a teratophthalmic embryo, while in the latter the fragment which eventually develops into an eye is all that survives of the germ. These effects are due mainly to two causes, namely to chemical alteration of the germ's substance which is greatest at the anterior end of the future embryo's body, and to increased osmotic (exosmotic) pressure on transfer to sea-water. The degree of resistance to the latter is the lowest at the point where the chemical alteration has been the greatest. Owing to this circumstance, apparently, it is from this part of the germ's substance that small fragments become occasionally split off by the pressure and shifted away from the potential embryo's position on the yolk. The increased osmotic pressure probably destroys entirely some eggs, for shortly after transfer of the surviving eggs to pure sea-water a number of them can usually be found to be dead. In some instances, however, small parts of such an egg which suffered great destruction from osmotic pressure may survive. This fragment, occasionally, by its differentiation into a solitary eye betrays its origin from the anterior portion of the potential medullary plate.

In commenting on the case which he reports, Loeb (l.c.) remarks that "the analogy with teratomata is obvious." Regarding this point I fully agree with Loeb, although teratomata could not by any means owe their origin to degeneration of an already formed embryo. They develop from fragments of the embryonic primordium which has sustained (blastolytic) injuries before the differentiation of tissues and organs. This point I have very recently ascertained by observation on living eggs, and I can fully confirm the correctness of Roux's ('95) generalizations on the subject. The latter author discussing the significance of some monsters recorded in the teratological literature arrives at the following conclusions (p. 205):

> Die Ursachen dieser hochgradigen Defect-und auch zum Theil der grossen Deformationsmissbildungen wirken, so viel wir jetzt sehen, schon zu Zeiten, ehe die einzelnen Organe und Gewebe vollkommen angelegt oder ausgebildet sind; daraus folgt, dass *die betreffenden Theile des Embryo 'nach' so hochgradigen Störungen ihrer selbst oder ihrer Nachbarschaft* nicht blos noch am Leben zu bleiben, sodern sogar *in einer oft noch an das Normale erinnernden Weise sich formal und geweblich zu differenziren vermögen.*

And further on, commenting on the capacity of small parts of an embryo to develop and to differentiate, Roux thus says (p. 207):

> Wir ersehen aus den angeführten Beispielen, dass *viele 'Theile' des Embryo unter günstigen Ernährungsumständen sich unabbängig von ihrer*

> *näheren oder ferneren Umgebung geweblich und formal zu differenziren vermögen, und dass dies sogar in annähernd normaler Weise geschehen kann.* Daraus geht hervor, *dass die Differenzierung dieser Theile an sich nicht eine Function der Wechselwirkung zwischen ihnen und den andern Theilen ist.*

These views of Roux apply very well to a great many teratomata which I have recorded, and among them the 'solitary eye' and the 'isolated eye' as well as to the case described by Loeb, to which I have referred above.

The 'solitary eye' and the 'isolated eye' are experimentally produced teratomata analogous to some well known spontaneous teratomata (e.g., 'dermoid cysts') in which hairs, skin, glands, and jaw fragments with well formed teeth can be found. According to Schwalbe ('07, part II, p. 99) eye parts have been repeatedly found in teratomata. Both Roux and Schwalbe regard such formations as striking evidence for the great capacity for self-differentiation possessed by parts of the embryo. In the light of this well supported view the morphogenesis of teratomata loses all of the mystery which has so long surrounded it. It is certainly not more difficult to comprehend than the differentiation of neurones from a minute fragment of medullary plate tissue explanted in Harrison's ('07, '10) pioneer experiment into a favorable nutritive medium (lymph). In both cases fragments of a primordium survive and differentiate fully, if nutritive and other conditions necessary for the continuation of life remain essentially unaltered.

It follows from what has been said that the morphogenesis of some teratomata ('dermoid cysts,' 'solitary eye,' 'isolated eye') can be accounted for only by a very high ability for 'self-differentiation' of metablastolytic fragments of the embryonic primordium. The conclusion is also evident that at a very early stage of development (coincident probably with the teratogenetic time limit) the parts of the embryonic primordium are already very sharply predetermined in regard to their histogenetic and organogenetic potencies.

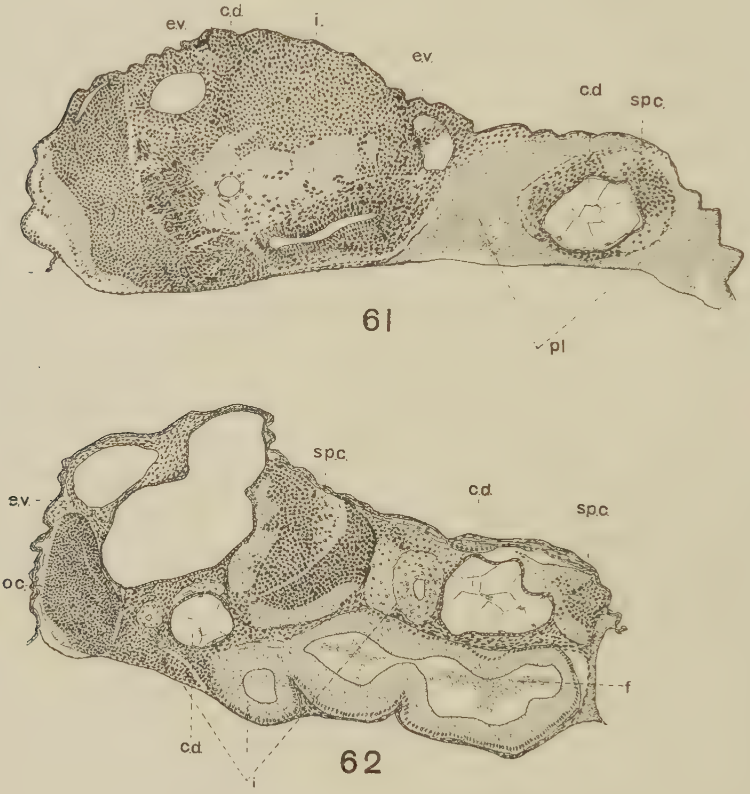
F. THE MICROSCOPIC ANATOMY OF SOME AMORPHOUS MONSTERS

As has been stated in a preceding chapter (cf. p. 498) these products of pathological development, due to treatment of eggs with butyric acid or acetone, resemble, when examined in toto, very much the amorphous monsters of man. Schwalbe ('07) has already pointed out that anatomical examination of the latter, often discloses conditions which are of interest from the onto-mechanical point of view. Bearing in mind the relative rarity of the spontaneous occurrence of such monsters, an anatomical investigation of the amorphous monsters recorded in my observations would appear to be desirable. My study of these embryos, however, being as yet incomplete, I shall confine myself for the present time to the following description of three such monsters with a view of returning to the subject in a later publication.

In figure 44 is seen a very misshapen embryo which makes the impression of coalesced, greatly malformed twins.

Examination of the entire series of sections, however, proves that the appearance of the embryo in toto is grossly misleading. For the case is not one of coalescence of two embryos, but rather of a peculiar disarrangement and distortion of parts of one embryo's body.

In the first sections there appears the deformed head of an embryo and a transversely cut tail in which the notochord only can be distinctly recognized. Between the head and the tail and partly under the head there is between the yolk and yolk-sac a space which is filled with a plasma-like mass. The brain of the embryo is greatly deformed and unilobed. One of the eyes has developed in a rather rudimentary manner; its retina and lens, however, are well differentiated. The other eye is lacking. Only a well developed lens can be seen on this side, and in some sections the neighboring nervous tissue, arranged in a cup-like manner around the lens, exhibits some resemblance to a retina in the earliest stages of differentiation. At this level there can be seen in the adjacent cross-sectioned tail, besides the notochord, also transversely cut coils of the intestine. No spinal cord, however, is yet seen in these sections of the tail, while in more posterior sections the notochord of the tail-part appears to be practically surrounded by the spinal cord (fig. 61). At the same level parts of the semi-circular canals come into view and also a part of an obliquely cut gut-coil. A few sections more posteriorly the plasma-filled space between the two parts of the embryo has disappeared from view and its place is now taken up largely by the obliquely sectioned intestine which stretches across between the two parts. The latter are now continuous. The spinal cord of the tail-part is now restricted to the region dorsal to the notochord and can be



Figs. 61 and 62 Camera lucida drawings of embryo in figure 44. *e.v.*, ear vesicles; *br.*, brain; *sp.c.*, spinal cord; *c.d.*, notochord; *o.c.*, optic cup; *i.*, intestine; *pl.*, plasma; *f.*, fibrin. × 100.

observed to gradually assume a shape approaching the normal. At this level one of the ear vesicles has disappeared from view while that of the other side (a very large one) is very prominent in the section. Laterally and ventrally from this ear vesicle (fig. 62) is seen a rudimentary optic cup. In the last sections the spinal cord runs across between the two parts of the embryo. It is particularly in these sections that the true nature of the monster is disclosed. From them we see that the posterior half of the embryo's body is in relation to the embryo's longitudinal axis turned at an angle of almost 180°. An oedematous blastodermic cavity filled with plasma and extending between the two so disarranged parts of the embryo has given the latter the appearance in toto of coalesced very malformed twins.

What is, now, the probable morphogenesis of this monster? The presence of one eye only would, according to what has been stated in the preceding, point to blastolytic injury sustained by the early embryonic primordium. This injury seems to be evidenced by the following facts. The ophthalmoblastic material of one side has, owing to chemical alteration, been somewhat inhibited in development and has given rise to an imperfect eye. The ophthalmoblastic cells of the other side have suffered great destruction (owing to increased osmotic pressure after chemical alteration?) and a remnant of them has been fragmented into two parts. Of the latter one has made some initial steps in development (having attained the shape and to some degree the structure of an optic cup) and stimulated the development of a lens, while the other fragment has been dislocated and developed into a rudimentary optic cup at the posterior level of the ear vesicles. The latter are both deformed and oedematous. The deformities of the brain are such as to point to a hap-hazard regulation of a brain primordium after blastolytic lesion. The oedematous blastodermic cavity filled with plasma suggests that no continuous blood circulation existed, the failure of blood vessels to develop being due to chemical alteration—a condition which can be found nearly always in embryos which have sustained blastolytic injury of a high degree. The strange space relation of the posterior half of the body to the anterior would apparently indicate the action of osmotic pressure, which while not sufficiently great to cause a rupture of the two halves of the primordium, has only shifted one of them out of its normal position on the yolk. Briefly, it may be said, that the effect of blastolysis, due to chemical alteration combined with osmotic pressure, was in this case a highly deformed amorphous embryo, which as figure 44 shows, can in toto easily be mistaken for coalesced malformed twins.

To blastolysis by chemical alteration and increased osmotic pressure are evidently due also the conditions found in the following embryo now to be described.

As can be seen from figure 30 the embryo is very deformed and only slightly suggests the body form of a fish. The irregularity of form pertains particularly to the head and the trunk, which latter appears to be greatly distended. There is no clear indication of the true nature of the embryo, namely that it is, as examination of sections shows, a case of a partial, fused duplicity.

The most anterior (transverse) sections show an anophthalmic and sharply pointed, highly oedematous head. Between the head and the yolk-sac there is a space filled with plasma (fig. 85) which can be recognized as the enlarged, oedematous pericardial vesicle. A lens is seen situated laterally, but there is no trace of an optic cup. Practically at the same level with the lens there appears ventro-laterally to it a transversely sectioned tube-like structure (gut?) which can be followed only in eight sections of 7μ thickness. Following the sections posteriorwards the oedematous brain is seen to increase in size and to be highly deformed. Still more posteriorly, i.e. at the posterior level of the lens the brain mass makes the impression of two fused, malformed brains, there being between the components a distinct lamella.

In sections through this double brain mass there can be seen at a distance from the embryo a transversely cut fragment of nervous tissue which makes the impression of a deformed spinal cord. It corresponds to the tissue fragment (*tf.*,) in figure 30. Between this fragment, the embryo and the yolk, there is a cavity filled with a plasma-like mass (fig. 86).

A second tissue fragment (tf_2 of figure 30) can be seen in more posterior sections on the side of the yolk opposite the embryo. This fragment proves on careful examination to be a very rudimentary eye, of which only the lens can be recognized with certainty.

The larger one of the two brain components (*a*) is more oedematous and its staining reaction with haematoxylin is weaker than that of the other component (*b*). This fusion of two brains into one mass becomes very distinct in still more posterior sections where component *a* now gradually diminishes in size, until it entirely disappears from view while the component *b* has encroached upon its place in the section. Several sections further posteriorly (fig. 86) there is seen a large optic cup at the ventral part of the brain component *b*. Retinal cells (rods and cones) of this optic cup can be recognized in some sections. The following is the complete picture presented at this level. Almost half of the section through the embryo is taken up by brain component *b*. Laterally from it is seen a transversely cut notochord, while ventral to it is the optic cup, and ventrally from the latter are seen cross-sectioned coils of an intestine. In the intestines a fibrin-like mass can be noticed, which points to defects in the circulatory system. Following the series more posteriorly there can be seen gradually to appear the spinal cord above the notochord. It is at first on one side fused with brain component *b* while in sections at a more posterior level (fig. 87) it is separated from the latter by a mass of mesodermal cells (poorly differentiated myotomes?). In still further sections the brain component *b* disappears entirely while a large cavity lined with endothelial cells and filled with plasma is seen to follow it in the sections. The last sections are cross sections of a deformed tail.

At the outset of the description of this monster it has been stated that the conditions found on microscopic examination point to blastolysis as a factor underlying its formation. While of course, a complete analysis of the morphogenesis of this monster is practically impossible, the two extra-embryonic tissue fragments would seem to point decidedly to blastolytic action. Considerable difficulty, however, is presented by the interpretation of the double, fused brain. Here an additional assumption may be made, namely that fragments of the blastolyzed embryonic primordium have secondarily come to fuse owing to shifting caused by increased osmotic pressure. Such spatial distortions and shiftings can be recognized in many duplicities. In our present case this hap-hazard regulation and fusion of parts would seem to be evidenced by the unusual position of an optic cup on the ventral side of the posterior part of the brain (cf. fig. 86).

That amorphous monsters result from blastolytic injury of a high degree is well illustrated also by the anophthalmic and greatly malformed embryo in figure 31.

Besides the defects and malformations which are seen on examination of the embryo in toto more are disclosed when sections are examined. There is no trace of eyes, and the nervous system is lacking almost entirely. In transverse sections (fig. 88) through the region of the head only some muscles, connective tissue, cartilage (of maxillary or mandibular arches?) and very rudimentary, closely approximated ear vesicles can be seen. In a few, more posterior sections there can be seen what might be taken for a very faint suggestion of nervous tissue in a very poorly differentiated stage. It is evidently this exceedingly small remnant (only seven sections of 7μ thickness) of the nervous system that has given rise to sensory part of the vestigial ear vesicles. Somewhat further posteriorly transversely sectioned coils of the intestine come into view. No trace can be found of either the notochord or the spinal cord. The fins, which also come into view at this level, are oedematous and filled with plasma (fig. 89). A few sections further posteriorly there is seen ventrally from the intestine a large coelomic cavity containing some fibrin. This cavity enclosed by the body wall is practically all that can be seen in the last sections of the monster.

IV. CONCLUDING REMARKS

It has been shown in the foregoing that an almost endless variety of monstrosities can be produced in fish, if eggs in the very earliest stages of development are subjected to the action of some toxic products of pathologic metabolism. The monstrosities thus produced resemble very much those occurring spontaneously in man and in other mammals. In other words, effects of unknown processes occurring in nature have been practically duplicated on a very large scale by the laboratory experiment.

In the latter the initial cause is known, because controlled by the experimenter. Some further steps in the sequence of events can, as I have recently convinced myself, partly be observed, and partly deduced from anatomical examinations of great numbers of monsters. The results of the latter agree well with those of anatomical examinations of the spontaneous monsters of mammals. Provided that in nature the same or very similar causes are responsible for the origin of monsters, the whole problem of teratogenesis in nature is—theoretically at least—within the control of the experimenter.

On the other hand, the experimental results recorded by previous work in embryology and teratology all seem to point to the conclusion that of teratogenic factors there are many, indeed almost as many probably as there may be agents injurious to organic existence. On what basis then, may it properly be asked, can experiments be performed with the aim of control of the causes, if the latter ones can only be a few out of a very great number of possible ones?

The problem, while obviously beset with many difficulties, is, however, not quite as elusive as it would at first appear. For, while there is probably an endless number of factors which, if acting on the egg, might cause it to develop in an atypical manner, it is, for our purposes, necessary to consider only those which can reasonably be conceived as acting in the mammalian body under certain unusual conditions. The latter are, as we shall presently see, relatively few.

These factors must be either of a physical or of a chemical nature.

Of the physical factors which might interfere with the typical development in utero of mammals only pressure and an increase in temperature could be imagined. The former has, indeed, for a long time been regarded as the cause underlying the origin of monsters. The inadequacy of this mechanical theory of teratogenesis has been repeatedly pointed out, and notably by Mall (l.c.). Neither is it probable that such an increase in the temperature of a mammalian female as would yet allow it to survive could materially affect an ovum in utero.

If, after what has been said, we dismiss the possibility of physical factors as the underlying causes of atypical development, there would remain only the group of chemical factors. Of the latter the number of those which can reasonably be imagined to be present in the mammalian's body under certain conditions, is rather limited. They might be some extraneous poisonous substances which may chance to gain entrance into the human (or other mammalian) body, they could also be bacterial toxines, or, finally, they may be autogenous poisons of the body, such as some toxic products of a disturbed metabolism.

Of the extraneous poisons which may find a way into the body without killing the individual, but only exerting an injurious influence on it, or on a developing ovum contained in the latter's uterus, there are probably not many. Alcohol and some other drugs, to which individuals may habitually be addicted, would probably occupy an important place among these substances. Lead-poisoning and phosphorus-poisoning might perhaps also be considered under this heading. However, while in the light of recent data, there is hardly any doubt left regarding the deleterious effect of parental alcoholism on the offspring (cf. Stockard '12) *no cases of human monsters are yet known of an alcoholic parentage.* The same would apply also to the occupational diseases of lead-poisoning and phosphor-poisoning. It cannot be denied that these poisons probably are capable of altering the typical course of embryonic development, but, evidently, they seem to be acting in a degree not sufficient to cause the development to become monstrous. Or, possibly the strength of action of these poisons necessary to produce monsters might kill the parent or bring about its sterility, and thus, no occasion may exist for human ova to be under the influence of such strong action of these poisons as would very materially alter the course of their development. The conspicuous lack of data regarding the relation between alcoholism and 'industrial diseases' on the one hand and the occurrence of monsters in man on the other hand would seem to point to the correctness of our conclusions. Besides, many monsters similar to human monsters are found in other mammals (and in sauropsids) which are neither exposed to the dangers of alcoholism nor to those of 'industrial diseases.' These considerations have led me to believe that poisons such as alcohol and other drugs as well as poisons of the 'occupational diseases' only very rarely, if ever, may lead to monstrous development in nature. The percentage of monsters whose origin may be due to these causes must, at best, be regarded as so small, as to be practically negligible.

Let us now turn to the next known chemical modification of the mammalian body, namely to the toxines of infectious diseases. Is there any likelihood that such toxines, when present in the blood of a female, would have such a deleterious effect on the ovum in its uterus, as to seriously derange the course of its development? This question is as yet difficult to answer, for the existing data on the subject are too insufficient and too indefinite. But even if future researches should prove the probability of a deleterious effect of maternal infectious diseases on the embryo in utero, which at present seems doubtful, there would remain only one other great source of chemical alteration of the ovum's environment, namely the products of pathologic metabolism. Of the latter there is a considerable number known at the present time, and a better knowledge of the pathological chemistry of disturbances of, particularly, proteid metabolism may add to this number.

Already Förster ('65) has advanced the hypothetical view that chemical alteration of the maternal blood may be one of the causes underlying the origin of monsters. This view although —strangely enough—almost entirely overlooked is now, however, not insupportable. For, recent advances in pathological chemistry have made us familiar with the chemical changes which our organism is subject to under certain pathological conditions. At the same time a better insight has been gained into the close physiological relation existing between the developing embryo and the mother. Thus, in the light of our present knowledge we must admit that Förster's idea although at the time expressed in a rather vague manner, is characterized by great foresight. One gains this impression particularly from Wolff's ('13) excellent summary on the biological relationship between embryo and mother during pregnancy.

This relation is, according to Wolff, particularly intimate in man, for the nutrition of the embryo is directly from the mother's blood. It is also of especial interest in such cases where, owing to pathological conditions, some toxic products of metabolism accumulate in the mother's blood. That the embryo is influenced by such alterations of maternal blood, is, according to this author, very plainly evidenced by clinical as well as experimental pathology. Thus it has, for instance, been found that the fetus of a nephritic mother may suffer from oedema and ascites (Sitzenfrey '10). The same obtains also in the animal experiment, if pregnancy is induced in a nephrotomized female.

As a very interesting illustration of the intimacy of physiological relation between fetus and mother in man may be considered the observations recorded by Kehrer.[9] According to this author bile acids and bile pigments can in the animal experiment not cross the placental barrier and thus they never reach the embryo, while in man the observation can be made that children of women suffering from jaundice during pregnancy may have jaundice at birth.

Such data would seem to leave no doubt that the mammalian embryo is sometimes subject to influences of a disturbed maternal metabolism. Whether all products of a deranged metabolism will have a deleterious effect on the embryo is a matter which, of course, needs investigation. In the preceding I have shown that if two substances known to occur in disturbances or carbohydrate metabolism, viz. butyric acid and acetone, be allowed to act on fertilized teleost eggs, the latter will develop into various monsters very strikingly resembling the monsters of man and other mammals. Even teratomata have thus been produced. These results would seem to leave little room for doubt that mammalian monsters may often be due to a coinci-

[9] Quoted from Wolff ('14).

dence of pregnancy with at least such disturbances of metabolism as diabetes.

This coincidence has been found to be fatal to the embryo. Thus, according to Seitz ('13) diabetes melitus has for a long time been considered as causing sterility in women. It is quite possible, I think, that in such individuals the ovum, due to toxic changes, might be expelled soon after conception, that such early abortions might have been mistaken for menstruation and that while perhaps no sterility existed a similar effect has been mistaken for it.

For, to quote Seitz:

> . . . . erst als Duncan über mehrere Fälle von Gravidität bei Zuckerkrankheit berichtete, änderten sich die Ansichten und nach den vorliegenden Statistiken über insgesamt 427 Frauen in geschlechtsfähigen Alter darf man annehmen, dass bei rund 5 procent der diabetischen Frauen Schwangerschaft eintritt.

The influence of diabetes on the development of the offspring is according to Seitz very disastrous:

> Besonders ungünstig ist die Rückwirkung des Diabetes auf des *Kind.* Übereinstimmend berichten die Statistiken, dass rund 50 procent aller Kinder intrauterin zugrunde gehen und zwar kann der Tod des Kindes jederzeit eintreten, sowohl in den ersten Monaten der Schwangerschaft als auch noch gegen Ende. . . . . Ein weiterer Teil der Kinder wird frühzeitig und schwach geboren; wiederholt wurde auch Erkrankung an Polyurie, an kongenitalem Diabetes und an Hydrocephalus beobachtet.

The data quoted above are, obviously, insufficient and, coming, as they do, from clinicians, they leave us altogether in the dark regarding the morphological effects of maternal diabetes on the embryo. The desirability of comprehensive data regarding fetuses aborted by diabetic mothers as well as those suffering from other diseases of metabolism can not be urged enough upon physicians, as they may not infrequently chance to observe these experiments of nature. But even more desirable would seem to be experiments on mammals. Here the various pathological conditions of metabolism must be imitated by experiment as closely as possible, and the animals so diseased mated in various combinations. The difficulties of such investigations are numerous, even if the most important one did not exist, namely that the experimental pathology of metabolism is practically yet in its infancy. But such difficulties may perhaps in time be at least partially overcome. And while the complete solution of the problem of the causal factors underlying the origin of monsters may yet be distant, well planned experiments and careful analysis of results may at least furnish direct evidence for the correctness of our assumption that monstrous development is primarly due to parental metabolic toxaemia, an hypothesis which in view of the noted results of our experiments would seem to be well justified.

Our experiments have, besides, pointed out the direction for investigations into the morphogenesis of monsters. In the preceding pages I have shown that microscopic analysis of all monsters discloses evidence of an action on the egg treated with butyric acid and acetone, which tends to dissociate (or disrupt) the germ's substance. This action, blastolysis, is a complex component in the sense of Roux ('95), being the result of the collective action of a number of factors potent in a varying degree in all eggs.

Some observations made in experiments during the summer of 1915 seem to suggest that the most important direct factors whose action results in blastolysis are the toxic effect of the chemical modification of the environment and an increase in osmotic pressure mainly after transfer of the eggs to pure sea-water. The first materially alters or destroys parts of the germ, while the latter may be regarded as a very forceful dissociating agent which disrupts the injured germ. The alteration brought about by the action of butyric acid or acetone seems to be due to the solvent action of these acids. To these conclusions would seem to point the fact that some hours after transfer of the eggs from butyric acid or acetone into pure sea-water there could invariably be found a sediment at the bottom of the dish in which they were kept.

The sediment was of a slimy consistency in the dishes containing eggs which had been treated with butyric acid, while in the dishes into which acetone-treated eggs had been transferred the sediment made the impression of a granular precipitate. Chemical analysis of these sediments may eventually disclose the nature of the chemical alteration of the eggs treated with these organic acids.

One rather evident effect which they have on the egg is that they increase its permeability. For on transfer from the solution to pure sea-water the eggs, owing to increased imbibition of sea-water, swell to an unusual size; after some time, however, they return to approximately the normal size and occasionally some eggs may be found considerably below the size of the normal, untreated egg. This indicates that the increase in permeability has called forth an increase in endosmotic pressure (imbibition) which allowed enough sea-water to enter the egg to dilute the substances dissolved by the acids, and a subsequent increase in (ex)osmotic pressure (shrinkage) owing to which the dissolved substances have passed out of the eggs and formed the sediment on the bottom.

Besides these two factors (toxicity of the medium and osmotic pressure) which are directly concerned with blastolysis there seem to be some other factors, to which apparently is due the enormous variation in the blastolytic effect and consequently the variation in the morphological deviations from the typical development. While some observations have been made which may bear on the nature of these factors, I can make no definite suggestion at this time and the determination of these factors must be deferred to future experiments.

However, while the analysis of blastolytic action into its component factors must thus for the present time remain incomplete, the evidence of this action is indeed very striking in practically all deformed embryos of my experiments.

Considering the fact that all these terata have been produced by experimentally induced blastolysis from eggs which would in a normal environment have given rise to normal embryos, it would no longer seem to be necessary to assume 'germinal variation' as the cause underlying the origin of ophthalmic monsters and various duplicate twins, as this has been postulated by some authors and notably by v. Hippel ('09) and H. H. Wilder ('08). The latter author, led by the "symmetry and regularity in anatomical details" of some monsters (cyclopia, diplopagus) has come to look upon them as "beings as orderly and perfect in their development as are the usual and normal types of being." In contradistinction to deformed embryos (true monsters) he even proposes the term "Cosmobion (plural cosmobia)" to designate such 'regular' symmetrical monsters, the underlying primary cause of which he assumes to be germinal variation. Quite apart from the circumstance that the cause of 'germinal variation' would still have to be explained, its assumption would seem unnecessary, because it can be demonstrated that of any given batch of eggs of approximately the same (early) stage of development those left in a normal environment will develop into beings typical for their species, while those subjected to the influence of agents which induce blastolysis will develop into monsters of which some may come very near to Wilder's own standard of 'cosmobia.'[10]

In our experiments such monsters have developed under the blastolyzing influence of some products of pathologic metabolism which might be imagined to be acting on the mammalian ovum during its uterine as well as pre-uterine existence. In the latter case we would be presented with what might, in a restricted sense of the word, be called 'germinal variation.' This germinal deviation, however, being due to a pathological cause, it would seem unwarranted to regard the developmental products of such ova, no matter how symmetrical and well formed they might be, as 'cosmobia' ('orderly living beings'). Besides, it is not entirely improbable that true 'cosmobia' might be produced, if it only were possible to imitate exactly the environmental modifications which underlie the origin of monsters in nature. This degree of accuracy is, however, not yet attained in our experiments.

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[10] One cannot but admire the refinement of Wilder's morphological speculations. But, with all due respect for this accomplished morphological philosopher, it is difficult to accept his theory of cosmobia. For, being, as it is, of necessity based on the assumption of germinal variation (of an apathological nature) it leads to the pessimistic conclusion that the problem of the origin of monsters (or at least of 'symmetrical monsters'—'cosmobia') is beyond control.

The assumption of the action of parental metabolic toxaemia on the (uterine or pre-uterine) ovum might also reasonably be extended to the male germ cell, which in fertilizing a normal ovum might cause it to develop into a monster. In this case the chemically altered spermatozoon would probably act in a manner very similar to the germ cell of another species. For, as it will be remembered, Moenkhaus ('04) has demonstrated that if eggs of Fundulus heteroclitus be fertilized with the sperm of Menidia various deformities will result. This experiment has since been repeated by Loeb ('15) and myself[11] and most recently by Reagan and Thorington ('15). Foreign sperm has evidently a toxic effect on the egg and thus deranges its development from the typical course. In analogy, the same may be true for the chemically altered spermatozoon of the same species. That thus modified spermatozoa, if fertilizing normal ova, may cause them to develop into degenerate individuals appears to be probable from statistical data on children of male alcoholics (Stockard '12). Very recently Stockard ('14) has demonstrated that the progeny of experimentally alcoholized guinea pigs, which had been mated to a normal female, was degenerate and deformed, thus suggesting that the chemical injury sustained by the chromatic substance of the spermatozoa had a deleterious effect on the normal ova of healthy females. Since male individuals are just as subject to disturbances of metabolism (although possibly less frequently) it is not improbable that the sperm of males suffering from metabolic toxaemia may bring about abnormal development when fertilizing normal ova. The effects of this union of normal chromatin with chemically altered chromatin may even be unnoticeable in the first generation of offspring and appear in the second and further consecutive generations as in the above mentioned breeding experiments of Stockard with guinea pigs. This author even reports that the results of mating descendants from an alcoholized father and a healthy mother deteriorate markedly with each consecutive generation. These data are very important for our considerations. For, if toxic products of pathologic metabolism should have an injurious influence on the male sex-cells—and there is every reason to believe that they do have such an effect—the chance for a teratogenic influence of parental metabolic toxaemia on the offspring is greatly enhanced.

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[11] Not published.

Elsewhere I (Werber '15 b) have pointed out the bearing which these conclusions and the results of my experiments with products of metabolic toxaemia on the teleost ovum may have on some, so far elusive, problems of medicine. Not less evident is their possible significance for eugenics and the biology of the race.

From the point of view, however, of the embryologist and pathologist our hypothesis and the results of the present experimental study based on it would seem to offer a rational basis for the solution of the old problems of the etiology and the morphogenesis of terata occurring spontaneously in many animals and notably in man and other mammals.

These problems may, I think, now be, at least partly accessible to experimental control.

V. SUMMARY

1. Recent results of investigations in experimental embryology and teratology pointed to the conclusion that the primary causes underlying the origin of monsters in man and other mammals are of a chemical nature. They also suggested that in the latter, particularly, these striking deviations from the developmental norm are due to autogenous chemical modification of the parental blood during disturbances of metabolism.

2. On the basis of this hypothesis experiments were performed on fertilized eggs of Fundulus heteroclitus which were subjected to the action of some substances of certain metabolic toxaemias.

3. Positive results were obtained with particularly two substances, which occur in toxaemia due to disturbances of carbohydrate metabolism, namely butyric acid and acetone.

4. A very great variety of monsters has resulted from these experiments, analogous to human and other mammalian monsters. The deformities concern the eyes (cyclopia, synophthal-

mia, monophthalmia asymmetrica, and anophthalmia), the ear vesicles (rudimentary structure, or lacking, or presence of one vesicle only, or synotia), the olfactory pits, the mouth, the central nervous system, the heart and blood vessels, the fins (unpaired pectoral fins, absence of pectoral fins or all fins, club-tail, etc.), and body form.

5. Oedematous conditions were found in many embryos lacking a continuous system of blood circulation in various parts of the body, which were greatly distended and contained plasma or fibrin and, not infrequently, many lymphocytes. This condition of hydrops is most frequently found in the head. It may be intracerebral or extracerebral and suggests an analogy with the congenital internal and external hydrocephalus of man, which latter may perhaps also be due to developmental imperfections of the blood-vascular system.

6. Blastolytic action of the chemically modified environment is assumed as a morphogenetic principle common to all terata of these experiments. Blastolysis either destroys part or all of the germ's substance, or it may split off and disperse parts of the latter.

7. The nature of blastolysis is two-fold, namely chemical and osmotic.

a. *Chemical blastolysis is a process of chemical alteration (by solvent or precipitating or coagulating action) of the germ's substance. This alteration results in dissociation or disintegration of parts of the latter (defect), or, occasionally in a decrease of the germ's chemical capacity for development and differentiation (inhibition).*

b. *Osmotic blastolysis sets in while the eggs are under the influence of the toxic solutions employed and again (more so) on their transfer from these solutions to pure sea-water. It results from the increase of permeability which allows sea-water to enter the eggs. The imbibition of sea-water by the eggs which swell rapidly, calls forth a fragmentation of the germ and dispersion of its parts which at this stage are yet capable of further independent development and differentiation.*

8. *All eye terata (cyclopia, synophthalmia, monophthalmia lateralis, anophthalmia) are due to a defect, viz., to blastolytic elimination*

of a fragment of, either, ophthalmoblastic or potential interocular material and not to an inhibition as was held by Huschke and Dareste and is now postulated by Stockard.

Only such cases of anophthalmia, where on microscopic examination rudiments (ill-differentiated optic vesicles or cups) are found, form an exception to this rule. Here an inhibition is assumed, due to a decrease of the chemical capacity for development (chemical exhaustion).

9. The frequent occurrence in these experiments of terata of the eyes (or the anterior part of the head) only is regarded as being due to the highest degree of susceptibility of that part of the earliest embryonic primordium, which eventually becomes the embryo's anterior end (animal pole). This assumption supported by many data, is based on Child's discovery of a definite susceptibility gradient ('metabolic gradient'-Child) along the chief body axis of many animals from various phyla.

10. The numerous meroplasts recorded and especially such teratomata as the 'solitary eye' and the 'isolated eye' point to a very high degree of capability of parts of the embryonic primordium for independent development and differentiation (self-differentiation—Roux).

11. While the occurrence in these experiments of various duplicities would seem to point to a relatively high prospective potency of parts of the teleost egg, the latter decreases very rapidly, for at an early stage (the Randwulst-stage or thereabout) these parts are already specifically predetermined as early (undifferentiated) anlagen of some organs. If at this stage a fragment be eliminated the result will be a defect of a corresponding organ or part of the body. Accordingly the teratogenetic time limit must be regarded as very brief, and especially so in the case of duplicities.

12. The results obtained tend to justify the hypothesis on which the experiments were based, namely that parental metabolic toxaemia may be the cause, or, at least, the chief cause underlying the origin of monsters.

DECEMBER 24, 1915

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PLATE 1

EXPLANATION OF FIGURES

63 Photomicrograph of a transverse section through the eyes and forebrain of the embryo in figure 3. *fbr.*, unilobed small forebrain; *o.p.*, fused olfactory pit. × 116.

64 Photomicrograph of a more posterior section (region of optic lobes) of the same embryo. *o.l.*, optic lobes; *o.c.*, optic cross. × 116.

65 Photomicrograph of a transverse section through the eye of the cyclopean embryo in figure 5. *o.l.*, optic lobes. × 135.

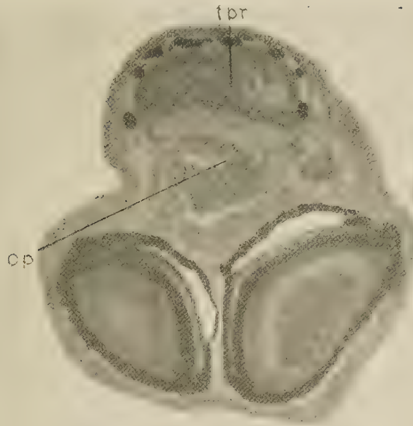
66 Photomicrograph of a transverse section through the eye of the cyclopean embryo in figure 12. *fbr.*, forebrain; *o.c.f.*, blastolytic optic cup fragment. × 116

67 A more posterior section (midbrain) of the same embryo, to demonstrate the large size of the fused eye and the small size of the defective midbrain. *mb.*, midbrain; *e.c.*, ear cartilage; *m.*, mouth. × 116.

68 Photomicrograph of a transverse section through the anterior part of the eye of the cyclopean embryo in figure 6. *p.*, pigment epithelium, *r.c.*, rods and cones; *o.p.*, olfactory pit; *br.*, brain. × 116.

69 A more posterior section through embryo in figure 6. *m.o.*, medulla oblongata; *oe.*, oedema; *pl.*, plasma of pericardium, into which the eye dips; *p.*, pigment epithelium; *r.*, retina. × 116.

70 Photomicrograph of a transverse section through the eye of the cyclopean embryo in figure 8, *fbr.*, forebrain; *y.*, yolk. × 116.



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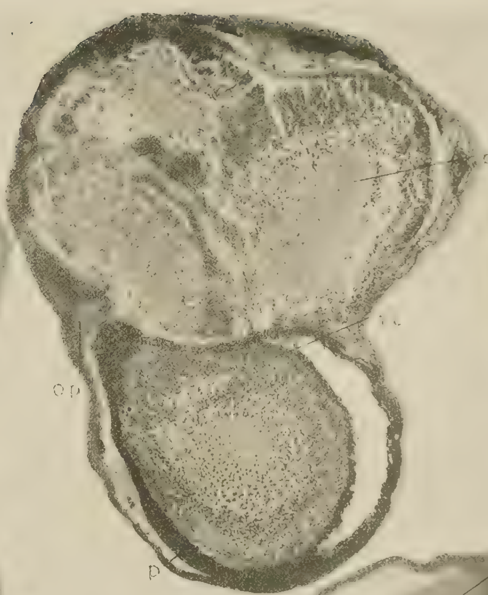

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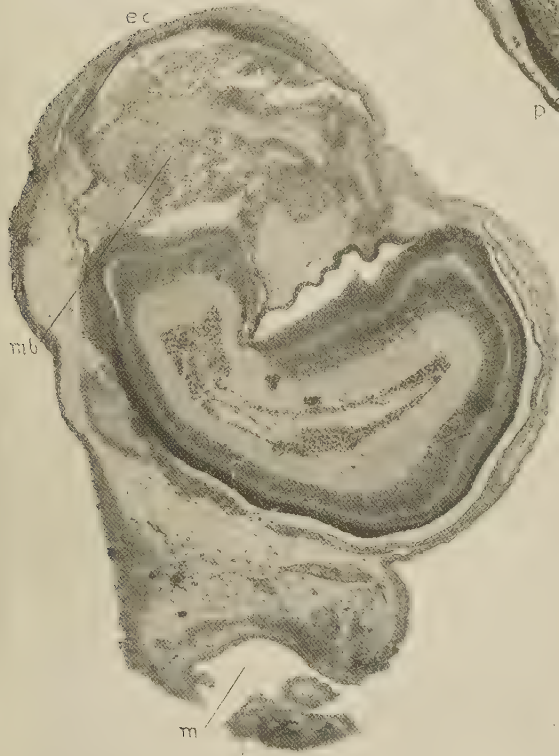

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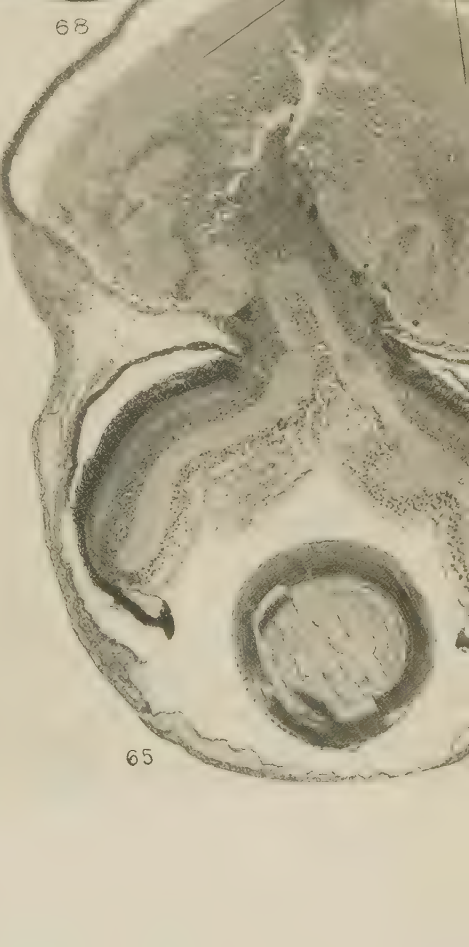

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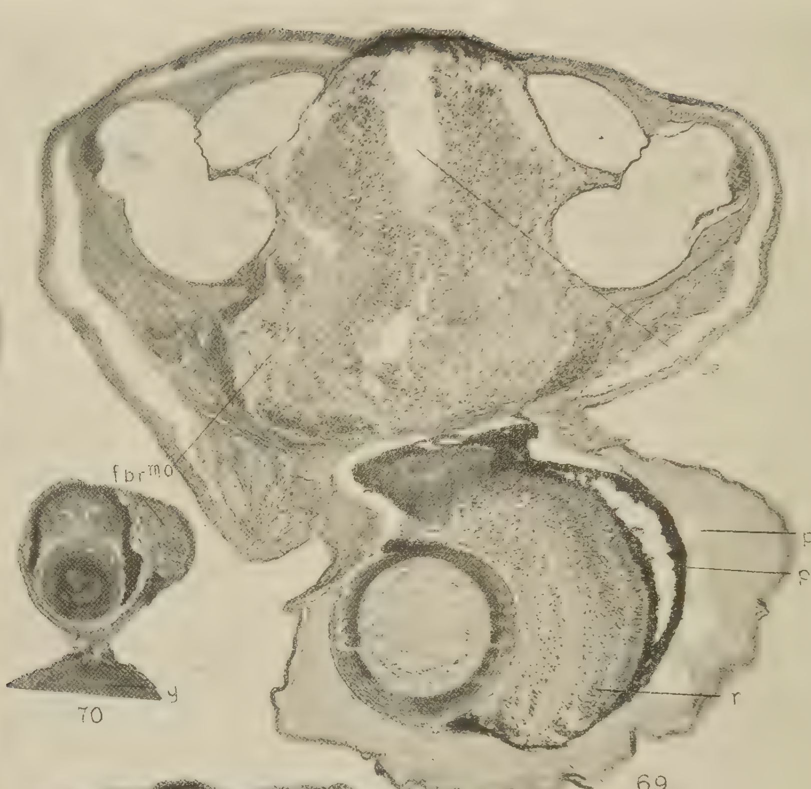

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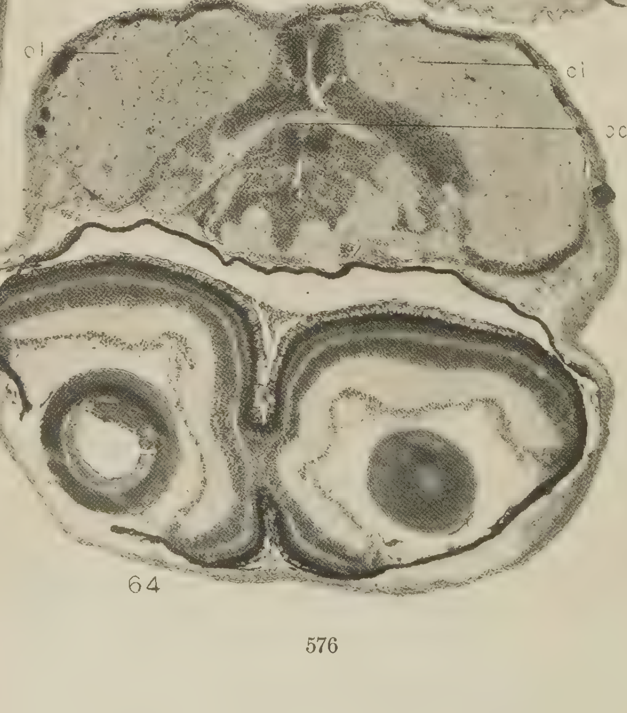

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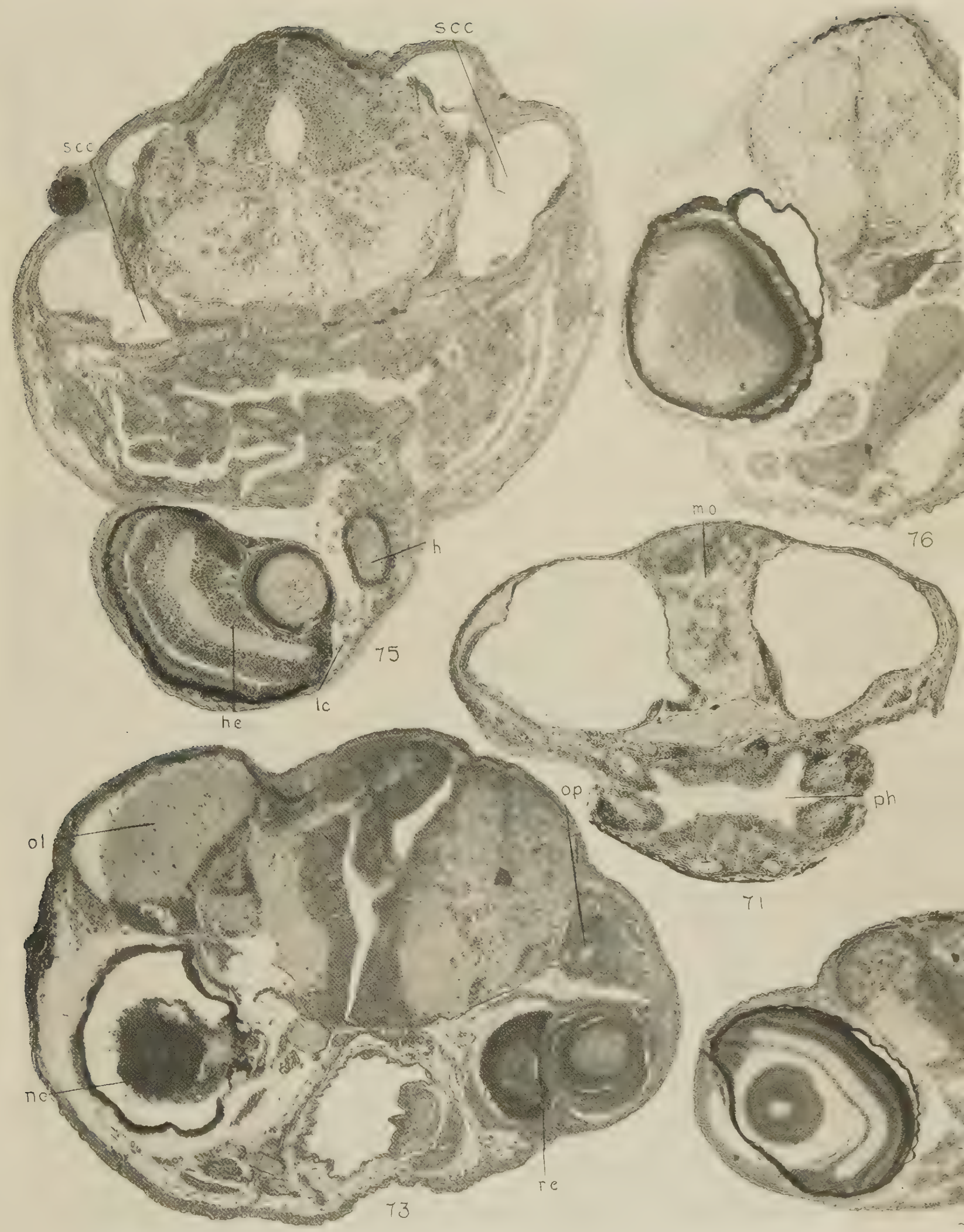
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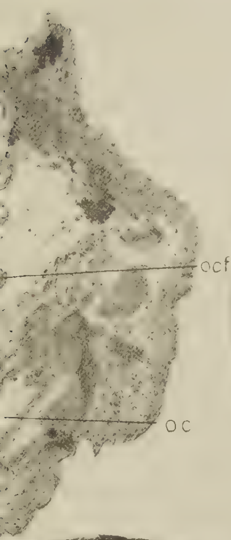

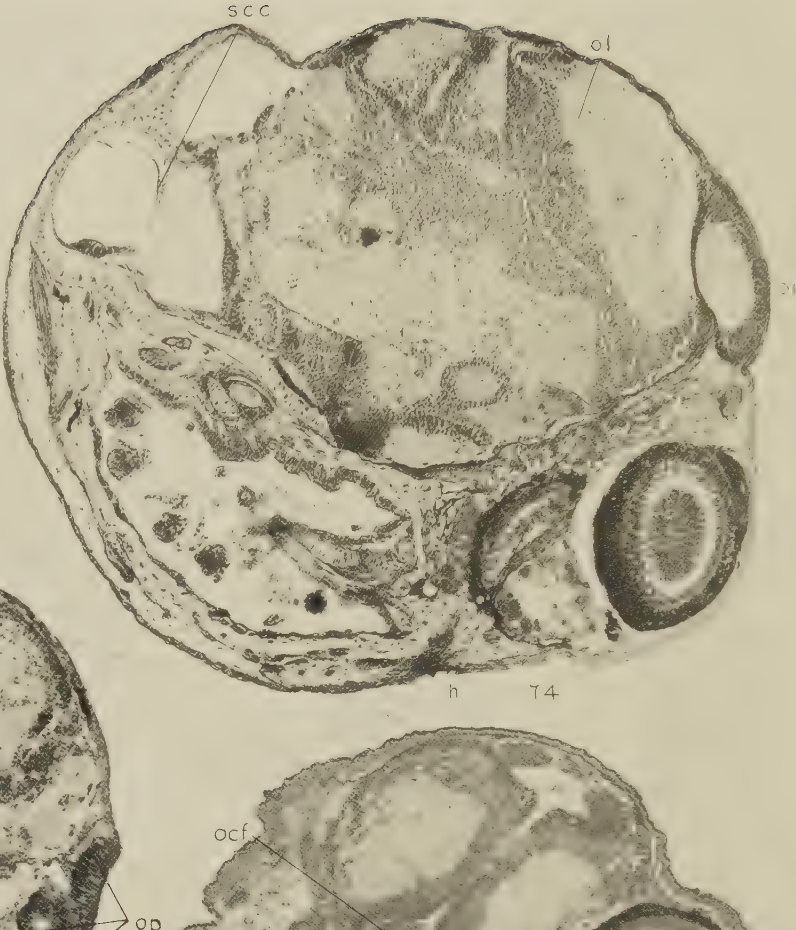

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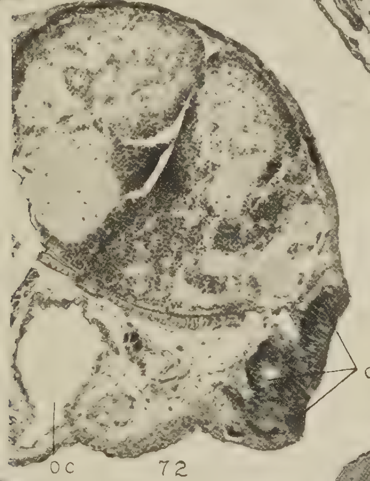

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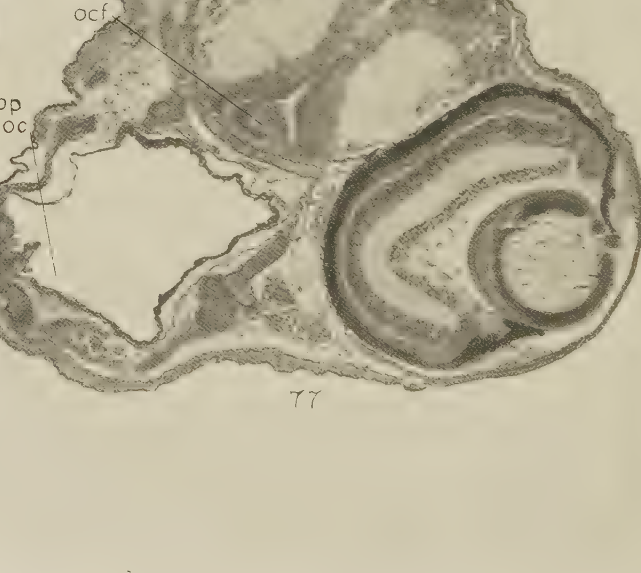

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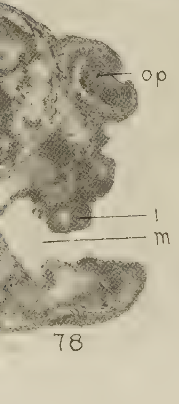

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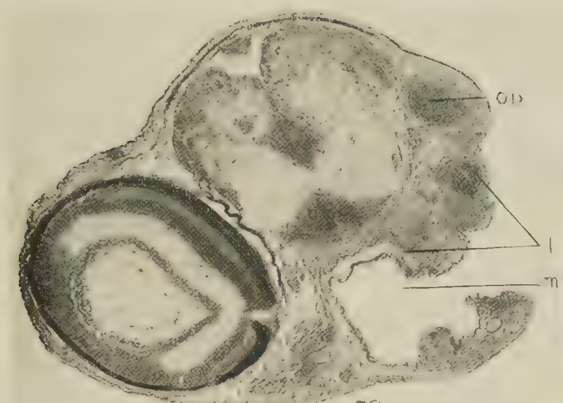

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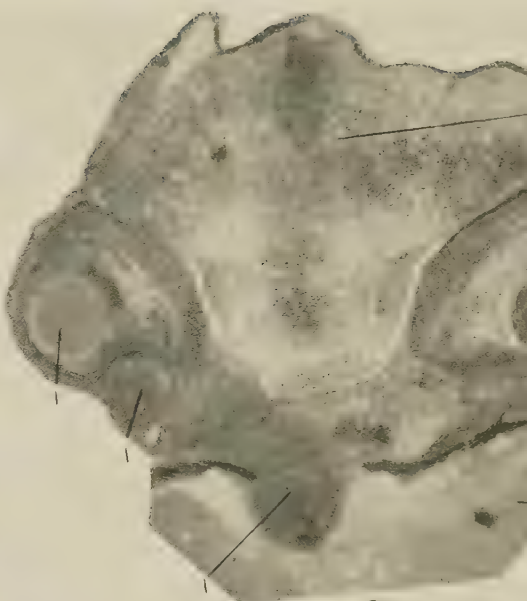

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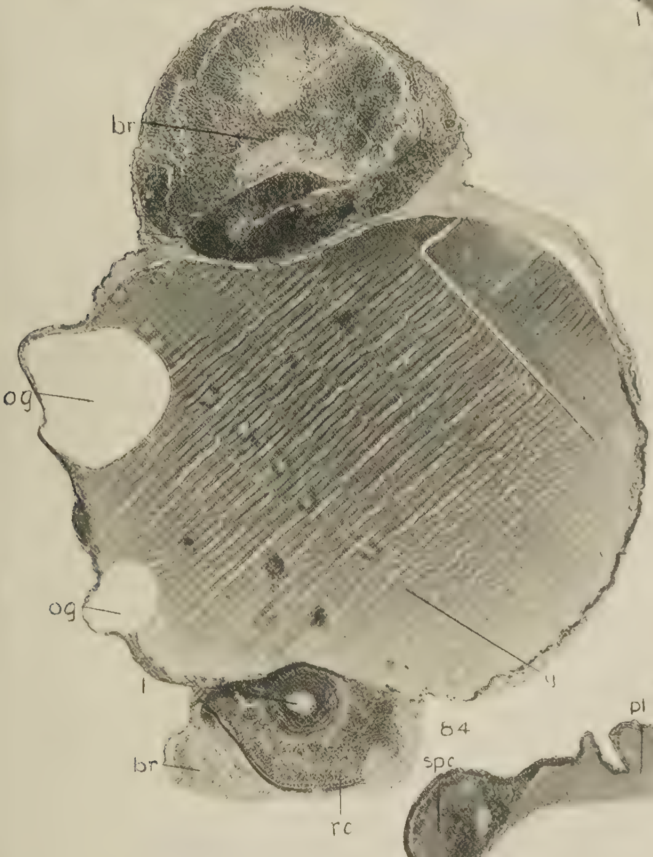

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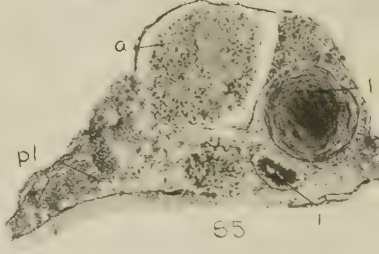

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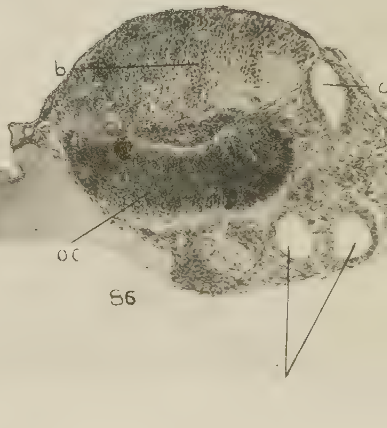

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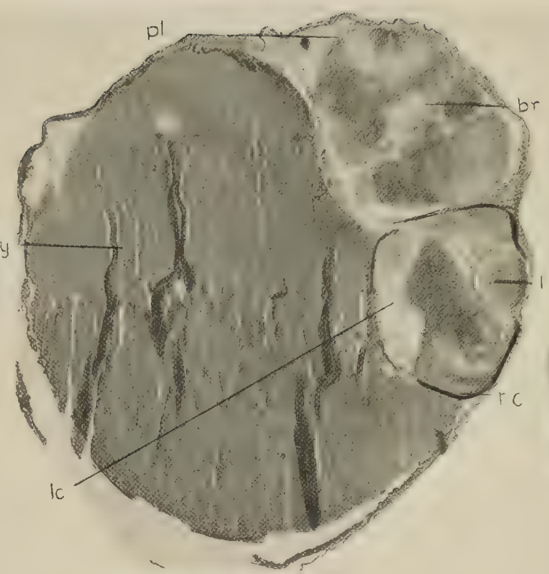

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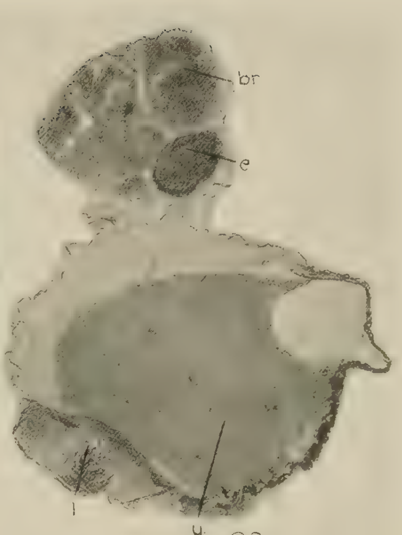

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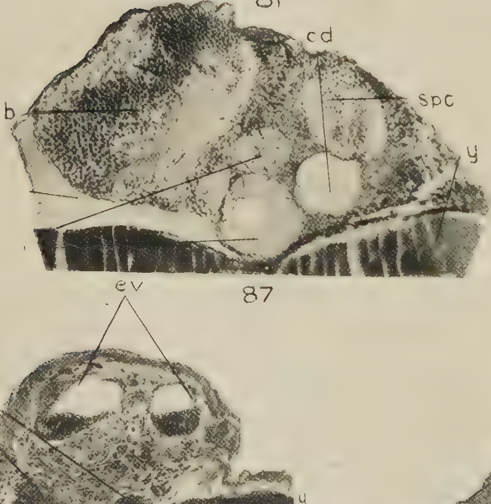

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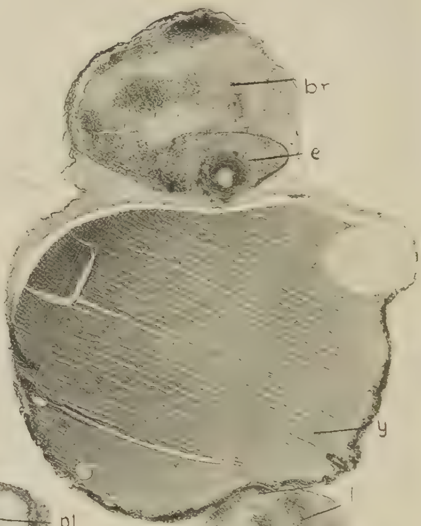

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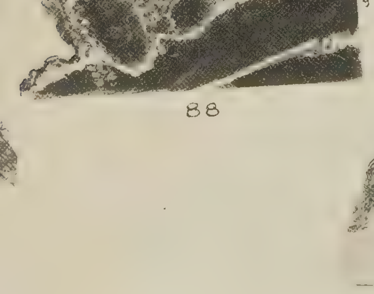

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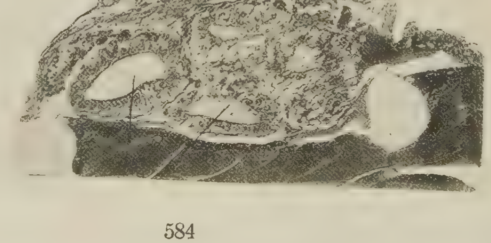

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THE PHYSIOLOGY OF THE ATRIO-VENTRICULAR CONNECTION IN THE TURTLE

I. Disturbances of A-V Conduction

HENRY LAURENS

From the Osborn Zoological Laboratory, Yale University

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INTRODUCTION

In a former paper (1) a functional differentiation in the A-V funnel of the lizard and turtle was demonstrated, in that the right and left parts of this funnel when reduced to a very narrow bridge could, either of them, conduct the contracting impulse from the A to the V, so that there was coördination between them. Furthermore it was found that if one of the parts of the connection were alone cut, there resulted a severe and long lasting block, while if both were cut permanent dissociation between A and V followed. In two later papers (2 and 3) the anatomical grounds for this physiological differentiation were shown to exist in a more intimate connection between the right and left funnel fibers and those of the inner wall of the ventricle than at other parts of the funnel.

After the publication of my first paper two others dealing with this same general subject appeared. Nakano (4) showed that the dorsal, right and left parts of the A-V funnel of the frog and salamander possessed a higher degree of conductivity than the remaining parts. And Mangold and Kato (5), from an analysis of the effects on the electrocardiogram of cutting and piercing the A-V connection in the fowl, have traced the pathways of the contracting impulse, which begin in the "sinus node" (6) and are collected at the level of the A-V boundary into a bundle, the principal branch of which goes into the right ventricle along the septum, while the other goes through the septum to the left and connects with the papillary muscles. Mangold (7) has given a summary of all these results.

To carry on the investigation of this problem of functional differentiation in the A-V connection a physiological and anatomical study of this region in the alligator's heart has been undertaken, the results of which will be published shortly.

Wishing to continue the investigation of this differentiation in the turtle heart it was necessary to repeat the earlier experiments that had been carried out on the heart of the lizard and of the turtle (Clemmys lutaria) on a native species. Malacoclemmys geographica was the species used.

EXPERIMENTAL

The methods of experimentation can be briefly described. The cervical cord was cut through and the brain and spinal cord destroyed. The plastron was then removed as rapidly and with as little loss of blood as possible and the heart laid free by slitting up the pericardium. Before and after opening the pericardium the frequency of beat was taken with a stop-watch. The heart was then suspended from the right auricle and the apex of the ventricle. In some cases the heart was excised, in others left in situ and a record of the "normal" heart taken for frequency of beat and length of A-V interval. To determine the parts of the funnel of greatest conductivity portions of this were cut through with a pair of fine scissors, one of two procedures in the manner of cutting being followed. Either a series of small cuts was made and after each a record taken to observe the effect of the cut on A-V conduction, and the cutting thus continued until an interference with the coördination of A and V took place, which was then allowed the opportunity of recovering, after which the process was repeated. Or a large portion of the funnel down to a desired narrow bridge of tissue was cut through at once, and a record taken to observe the resultant degree of block and its recovery.

It must at first be pointed out with regard to the study of the A-V conduction time, etc. that, as has been earlier noticed (1 and 4), every mechanical disturbance of the heart has some effect on its activity, which may express itself in a retardation or acceleration, or a short standstill of both A and V or of V alone, extra systoles, group formation, pulsus alternans, ventricular fibrillation, etc., all of which are of short duration and are not to be regarded as typical effects of the particular operation. Lewis (8) noted that once the pericardium is opened irregularities in the beat occur which he considers due to changes in temperature and in the position of the animal. Meek and Eyster(9) also note that handling the heart leads to irregularities.

RESULTS

Briefly stated, the results obtained by cutting through parts of the funnel of Malacoclemmys substantiate in the main those obtained with Clemmys. Following the plan of Nakano (4) attempts were made to divide the ring into eight parts, but it was soon found that by this nothing was to be gained. Particular attention was also paid to the dorsal portion of the connection, since Nakano had found that in the frog this was an important part, but with negative results.

The right and left parts of the funnel were again found to be possessed of a higher degree of conductivity than the other parts. But my former results with regard to the consequent marked and long lasting block when the one or the other of these parts was cut through, and the permanent dissociation produced when both were severed have not been obtained. For in Malacoclemmys the broad ventral and dorsal portions of the funnel remaining when the right and left sides are cut through are fully capable of conducting the contracting impulse so that the ventricle, after longer or shorter standstill, beats again, either in a low degree of partial block which shortly recovers, or immediately coördinated in a 1 : 1 rhythm. In other words no part of the connection in Malacoclemmys can be said to be indispensable for the coördinated conduction of the impulse to the V. But nevertheless, as in the heart of Clemmys, the right and left parts of the funnel are possessed of a higher degree of conductivity. For along either of them when all other parts are severed, and when they are reduced to a mere thread of tissue incapable of being further narrowed, the impulse from the A is able to reach the V and cause it to beat coördinated with it and in a normal 1 : 1 rhythm.

The cutting of any part of the connection increases the length of the A-V interval to a greater or less extent, but with apparently no reference to the portion severed (see table 1). In contrast to the relatively slight effects on lengthening the A-V interval when a particular portion or portions of the funnel are severed are those when all but a certain small part is cut through. Looking over these examples it will be apparent that in the heart of Malacoclemmys, as in Clemmys, the right and left parts of the funnel are the most functional, not only from the standpoint of it being possible to narrow them down more than the dorsal and ventral portions without affecting coördinated conduction or obliterating it entirely, but also from the standpoint that when narrowed down to so small a bridge of tissue that another cut

TABLE 1

Effects of cutting parts of the A-V connection on the length of the A-V interval

NO.	TEMPERATURE	TIME	PORTION CUT	LENGTH OF A-V INTERVAL	RETARDATION	FREQUENCY	REMARKS
7	19.5	10.35		1.12		18	
		10.38	Right and left, leaving broad ventral and dorsal connection	1.20	0.08	17	Short standstill of V, followed by 1 : 1
8	18.2	9.20		0.97		18	
		9.26	Right and left deeply	1.41	0.44	18	Short standstill of V, followed by 2 : 1, quickly 1 : 1
		9.28		1.31	0.34	17	
10	20.0	10.56		0.72		22	
		10.59	Right and left deeply	1.62	0.90	20	Short standstill of V, followed by 1 : 1
11	22.8	8.57		0.83		24	
		9.00	Right and left lightly	0.95	0.12	24	
		9.02	Right and left deeply	1.03	0.20	24	Short standstill of V, followed by 1 : 1
2	22.0	9.45		0.96		18	
		10.00	Right	0.96	0.00	18	
3	22.0	9.25		0.89		18	
		9.28	Left	0.94	0.05	17	
24	22.0	10.00		0.86		18	
		10.06	Left	0.94	0.08	18	
25	21.0	9.35		0.79		19	
		9.40	Dorsal	0.87	0.08	18	
5	21.2	10.24		1.04		18	
		10.28		1.08		16	
		10.30	All except left				Partial block, recovery to 1 : 1
		10.50		1.62	0.54	17	
3	22.0	9.20		0.88		18	
		9.25		0.89		17	
		9.28	Left	0.94	0.05	17	
		9.30	All except narrow bridge on right	1.62	0.68	17	Short partial block, followed by 1 : 1

TABLE 1—Continued

NO.	TEMPERATURE	TIME	PORTION CUT	LENGTH OF A-V INTERVAL	RETARDATION	FREQUENCY	REMARKS
6	18.3	9.20		0.95		21	
		9.23 to 9.44	A series of cuts leaving bridge on dorsal	1.88	0.93	20	
		9.45		1.93		20	At 9.46 irregular dropping of V systoles, ending in dissociation
12	20.9	9.22		0.99		24	
		9.26	Right and left lightly	1.08	0.09	24	
		9.28 to 9.33	All remaining except dorsal	1.60	0.72	24	Fibrillation of V, followed by 1 : 1
7	19.5	10.35		1.12		18	
		10.38	Right and left lightly	1.20	0.08	17	
		10.42	Dorsal, leaving ventral			18	Standstill of V for three minutes, then 4 : 1, 2 : 1 and at 10.46 1 : 1
		10.46		1.32	0.20	18	
		10.50	Ventral bridge narrowed			18	Permanent block

would necessarily sever it the A-V interval is not lengthened so much on the average as when the dorsal or ventral portion of the funnel alone remains.

It is clear that whether and to what degree a retardation of the A-V interval will take place depends not merely upon general injury, such as the length of the experiment and the number and extent of the cuts made, but also upon the importance for A-V impulse conduction of the part injured. The width of the bridge of tissue is less important for coördinated conduction than the part that has been left. There is an independence also between the ability to conduct and the velocity of conduction. For whenever a partial block has been produced which later disappears the A-V interval is longer than it was before the block occurred.

Gaskell (10) from a few experiments, which were far from complete or conclusive, on cutting away various parts of the A-V connection, stated that the right ventral portion of the connection just under the aorta ("the upper portion of the right ring") was the most important part in the conduction of the contracting impulse from A to V. (Although there is no connection here between the musculature of the A and V, since the funnel musculature goes over into that of the bulbus (2 and 3)). Meek and Eyster (9) have found that the impulse in going from the A to the V reaches the various parts of the ring in the following order: anterior part, left part, right part. They call attention to the similarity between their results and those of Gaskell, and also believe that they have substantiated the old belief that the contracting impulse is of the nature of a wave which sweeps over the sinus and auricles and spreads downward from the circular fibers of the A-V ring over the V to terminate at the apex. Negativity in the basal portions coming first is supposed to be largely determined by proximity to the ring, although they do not exclude the possibility of there being certain pathways along which the wave may pass more directly or with greater ease, as shown by their results on the earlier negativity of the left part.

Somewhat opposed to these results are those of Lewis (8) obtained with the heart of Testudo graeca. According to him the distribution of the excitation process over the ventral surface of the V is that, as a rule, the base and central region, or the central, is earliest and the apex the latest. Sometimes he obtained a high reading from the region of the base in the neighborhood of the truncus arteriosus and sometimes the right margin was found to be activated at a later time than the left. The lateral and posterior surfaces of the heart showed the same distribution. From a comparison of readings taken from the inner surface of the V and compared with those from the outer, Lewis comes to the conclusion that there is in the V no special tissue which conducts more rapidly than the surface muscle fiber, as he found also to be the case in the heart of the toad. It is interesting to note that he obtained, table 4, p. 233, in the toad the earliest internal reading near the ring in front at the left auricular border, and that on p. 243 he makes the statement that the earliest internal readings are taken from the portion of the lining which borders the A-V ring, though he does not state just what part of the ring is the earliest. It remains to consider Lewis' idea of the manner in which the impulse from the auricles is communicated to the V (see his fig. 6, p. 236). He regards it not as in the nature of a simple surface spread, but, as in the V of the dog, a distribution from within outwards from the ring to the surface through the wall of the heart. "The musculature of the A and V do not meet at the surface of the A-V line but the ring is prolonged as a tube for some distance into the V; passing downwards it meets and fuses with the processes of muscle which radiate towards the central surface zone and apex of the heart. The trabeculae first carry the excitation to the central surface zone of the heart. Later the wave courses farther toward the apex and at the same time mounts to the base."

The results of these investigators are given to point out the want of agreement which exists between them, and how they both differ from what has been found by the method of severing portions of the A-V funnel. Perhaps a word more may be added regarding the arrangement of the fibers of the funnel. In the first place the physiological differentiation in the right and left portions of the funnel has been explained, (2 and 3), by the closer connection here. On the other hand the ventral and dorsal parts of the funnel are the first to come into union with the fibers of the ventricle and we might therefore expect these portions of the ventricle to become negative earlier. But Lewis finds that the same thing occurs on the lateral surface of the ventricle, so that it may be said that the places of earliest negativity are where the funnel musculature fuses with that of the ventricle. From Lewis' diagram and tables it can be seen that the heart of the tortoise differs from that of the toad in that the excitation wave reaches the basal portion of the surface of the heart of the tortoise before it reaches the apex, while the surface basal muscle of the toad heart is the last to be activated. This is in all probability due to the reason which Lewis (p. 246) gives for this chief difference between the distribution of the excitation process in the toad's heart and in the tortoise heart, namely, that in the tortoise ventricle the fusion of the ring musculature with the ventricular wall takes place at a level relatively nearer to the base than in the toad's heart.

It would be completing a detail of this phase of the problem of A-V conduction to study tracings of the passage of the wave of negativity before and after cuts of the kind above described were made. This would undoubtedly bring out more clearly the effects of the severing of this or that part of the funnel and the effects on the velocity of conduction between A and V.

DISTURBANCES OF A-V CONDUCTION

The disturbances of A-V conduction which are brought about by means of cutting the connection are of considerable general interest. As earlier stated with every cut made through a part of the A-V funnel there is, as a rule, an increase in the length of the A-V interval, without necessarily producing a condition of block (see table 2). This increase either becomes spontaneously greater or remains constant, and even in a few cases with the improvement in conduction decreases.

LENGTHENING OF A-V INTERVAL LEADING TO PARTIAL BLOCK

Of much greater interest than the mere slowing of conduction between A and V are those cases in which the retardation leads to the production of block (partial). The various degrees of such disturbances can be observed with great clearness in the turtle heart. A short description accompanied by curves and tables will now be given of them.

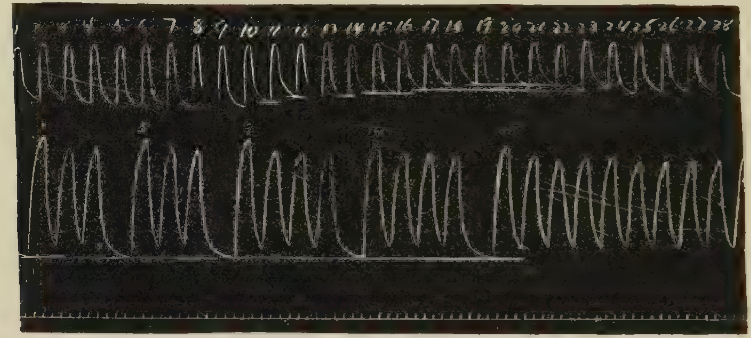

Fig. 1. Partial block characterized by gradual increase in length of A-V interval.

As is well known one of the types of partial block is characterized by a gradual increase, with every heart cycle, of the A-V interval until a contraction of the V is missed (11 and 12). During the pause the V, or the connecting system, recovers and the next cycle has a shorter A-V interval, which with every successive cycle again increases until another V systole is missed and thus the process is repeated. In figure 1 an example of such a type of block is shown and its final recovery to a normal 1 : 1 rhythm. The lengths of the A-V intervals are given in table 3 under No. 18 a. It will be observed that at the end where

TABLE 2

Effects of continued cutting of the A-V connection on the length of the A-V interval

NO.	TIME	PORTION CUT	LENGTH OF A-V INTERVAL	FREQUENCY	REMARKS
3	9.20		0.88	18	
	9.25		0.89	17	
	9.28	Dorsal ligament and small cut on left......	0.94	17	
	9.30	All except a bridge on right................	1.62	17	
	9.40		1.60	16	
	9.50	Right bridge narrowed	1.71	16	Rhythm = 2 : 1
	9.53		2.09	16	Rhythm = 1 : 1
	9.58	Right bridge narrowed		16	Standstill of V for 2 minutes, followed by 3 : 1, recovery to 2 : 1
	10.26		1.77	15	Rhythm = 2 : 1
	11.20		1.79	12	Rhythm = 1 : 1
6	9.20		0.95	21	
	9.23	Right..................	1.06	22	
	9.25	Left...................	1.31	21	
	9.29	Right and left more deeply on dorsal.....	1.51	20	
	9.35	Ventral...............	1.74	20	
	9.41	Septum...............	1.76	20	
	9.44	Dorsal slightly narrowed...............	1.88	20	
	9.45		1.93	20	
	9.46			20	Block, and finally dissociation
8	9.20		0.97	18	
	9.26	Right and left deeply..	1.41	18	Short standstill of V, 2:1 rhythm of short duration, 1 : 1
	9.28		1.31	17	
	9.30	Ventral entirely through.............	1.71	16	
	9.34		1.60	16	
	9.38	Dorsal slightly from right................	1.71	16	
	9.44	Dorsal slightly from right................	1.62	15	Rhythm = 2 : 1
	9.45		1.91	15	Rhythm = 1 : 1
	9.50		1.71	15	
	10.09		1.77	15	
	10.11	Dorsal narrowed from left..................		15	Standstill of V, later an independent V rhythm

TABLE 2—Continued

NO.	TIME	PORTION CUT	LENGTH OF A-V INTERVAL	FREQUENCY	REMARKS
10	10.56		0.72	22	
	10.59	Right and left deeply	1.62	20	Short standstill of V, followed by 1 : 1
	11.03	Dorsal..................	1.39	21	Rhythm = 4: 1
	11.06		1.39	22	Rhythm = 2: 1
	11.07		1.85	22	Rhythm = 1: 1
	11.15		1.59	22	Rhythm = 1: 1
	11.18	Ventral narrowed......		22	V is still
11	8.57		0.83	24	
	9.00	Right and left lightly	0.95	24	
	9.02	Right and left deeper	1.03	24	Short standstill of V, followed by 1: 1
	9.04	Dorsal and ventral from left.............		24	Dissociation
	9.07		1.48	24	Rhythm = 1: 1
12	9.22		0.99	24	
	9.26	Right and left lightly..	1.08	24	
	9.28	Right and left deeper..	1.36	24	
	9.32	Ventral.................		24	Fibrillation of V, followed by 1 : 1 after a single large V_s
	9.33		1.60	24	

the block disappears the A-V interval has a length almost equal to that after which the V systole was in the habit of falling out. In all figures the upper tracing is of the contractions of the right auricle, the middle those of the ventricle. The bottom line is time in seconds.

Figure 2 illustrates the influence of A rate and irregularities in A beat in the formation of this type of block. A block between A and V had been caused at 9.54 by cutting through a part of the funnel. The remaining funnel fibers gradually recovered from the injury, the V beginning by giving single, isolated contractions at 9.55, and at 9.57 suddenly beginning to beat in a 4 : 1 rhythm. At 9.59 this became 2 : 1 and continued regularly so until 10.04 at which time the record shown was taken. The A and V are for the most part beating in a 2 : 1 ratio, but occasionally, for several successive contractions, the rhythm is 1 : 1. When the lengths of the A-V intervals are measured (see table 4) under 10.04 it is seen that they are of a constant value, 0.86 seconds (0.8 second has been added since the A lever is that much

TABLE 3

Lengths of A-V interval in cases of partial block. Note the increase in length of interval when the rhythm becomes 1 : 1

NO. 18 A		NO. 10		NO. 11		NO. 20		NO. 18 B		NO. 4		NO. 14	
A_s	A-V	A_s	A-V	A_s	A-V	A_s	A-V	A_s	A-V	A_s	A-V	A_s	A-V
1	1.68	1	1.31	1	1.39	1	1.46	1	1.79	1	1.45	1	0.99
2	1.84	2		2		2		2	2.02	2		2	
3	2.01	3	1.31	3	1.39	3	1.46	3		3	1.45	3	0.99
4		4		4		4		4	1.79	4		4	
5	1.68	5	1.31	5	1.39	5	1.46	5	2.02	5	1.08	5	0.99
6	1.90	6		6		6		6		6		6	1.20
7	1.96	7	1.31	7	1.39	7	1.46	7	1.79	7	1.22	7	
8		8	1.85	8	1.46	8		8	2.02	8	1.43	8	0.99
9	1.62	9	1.62	9	1.51	9	1.46	9	2.02	9	1.51	9	1.04
10	1.79	10	1.40	10	1.40	10	1.86	10		10	1.62	10	1.12
11	1.96	11	1.40	11	1.40	11	1.84			11		11	1.22
12	2.12	12	1.40	12	1.39	12	1.80			12	1.36	12	1.28
13						13	1.77			13	1.43	13	1.28
14	1.68					14	1.77			14	1.48	14	
15	1.84					15	1.68			15	1.52	15	1.04
16	1.96					16	1.74			16	1.48	16	1.16
17	2.12					17	1.68			17	1.52	17	1.28
18						18	1.68					18	1.28
19	1.74					19	1.71					19	1.16
20	1.96					20	1.68					20	1.16
21	1.96					21	1.71					21	1.16
22	1.96					22	1.68						
23	1.96					23	1.71						
24	1.96					24	1.68						
						25	1.65						
						26	1.60						
						27	1.60						

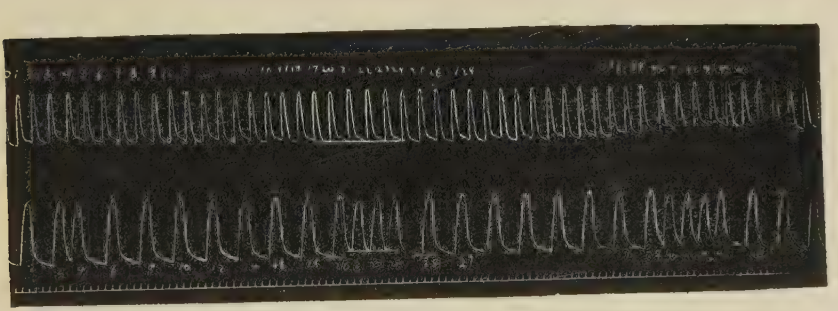

Fig. 2. Influence of A rate on partial block.

TABLE 4

Influence of A rate on formation of partial block (cf. figs. 2, 3 and 4)

10.04			10.10			10.22		
As	A–V	A–A	A_s	A–V	A–A	A_s	A–V	A–A
1	0.79		1	0.97		1	1.16	
		2.08			2.43			2.49
2			2	0.97		2	1.16	
		2.38			2.43			2.66
3	0.86		3	0.97		3	1.08	
		2.52			2.35			2.99
4	0.86		4			4	1.16	
		2.30			2.43			2.82
5			5	0.97		5	1.08	
		2.38			2.27			2.66
6	0.86		6			6	1.16	
		2.30			2.27			2.57
7			7	0.97		7	1.02	
		2.38			2.43			2.49
8	0.86		8			8	1.08	
		2.38			2.35			2.49
9			9	0.97		9	1.16	
		2.38			2.35			2.57
10	0.86		10			10	1.16	
		2.16			2.43			2.91
11			11	0.97		11	1.02	
		2.30			2.99			2.91
12	0.86		12	0.97		12	1.02	
		2.16			2.75			2.66
13			13	0.97		13	1.02	
		2.23			2.59			2.49
14	0.86		14	0.97		14	1.02	
		2.16			2.43			2.41
15			15	0.97		15	1.02	
		2.16			2.51			2.41
16	0.86		16	0.97		16	1.02	
		2.16			2.51			2.91
17			17	0.97		17	1.02	
		2.16			2.48			2.99
		2.23	18	0.97		18	1.02	
					2.43			2.74
18	0.86		19			19	1.02	
		2.16			2.43			2.49
19			20	0.90		20	1.02	
		2.16			2.43			2.49
20	0.86		21	0.97		21	1.02	
		2.66			2.51			2.49

TABLE 4—Continued

10.04			10.10			10.22		
A_s	A-V	A-A	A_s	A-V	A-A	A_s	A-V	A-A
21	0.86		22	0.95		22	1.02	
		2.66			2.43			2.41
22	0.86		23			23	1.02	
		2.52			2.43			2.99
23	0.86		24	0.90		24	1.02	
		2.38			2.51			2.66
24			25	0.95		25	1.02	
		2.30			2.43			
25	0.86		26					
		2.23			2.43			
26			27	0.90				
		2.30			3.08			
27	0.86		28	0.95				
		2.16			3.08			
28			29	0.95				
					2.59			
			30	0.95				
					2.67			
37	0.86		31	0.95				
		2.08			2.59			
38			32	0.95				
		2.16			2.59			
39	0.86		33	0.95				
		2.66						
40								
		2.52						
41	0.86							
		2.38						
42								
		2.38						
43								
		2.30						
44								

too far to the right). The A-A interval is, however, variable and shows a gradual decrease in length after A pauses which occur more or less regularly. These A pauses, or sudden lengthenings of the A-A interval allow the A-V connection (or the V) time to recover between the A systoles so that the impulse reaches the V. Perhaps too the strength of the A impulse is greatest after the pause and decreases gradually as the rate increases. After the pause the rate of beat increases as evidenced by the gradual shortening of the A-A interval until finally the A-V rhythm becomes 2 : 1, the A-V connection being able to conduct only every other impulse, or the V to respond to only every other.

In figure 3 a later record of the same heart is shown taken at 10.10 (see table 4). The block has improved so that the rhythm is for the most part 1 : 1. The A-V interval has increased somewhat being now 0.97 second and constant (0.7 second has been added). The rate of A has decreased, being now about 25 per minute. Here the same tendency on the part of the rate to gradually increase is manifest so that the rhythm either becomes 2 : 1 or a V systole is missed. Again the pause, lengthening the A-A interval, lets the impulse through every time and the rhythm becomes 1 : 1 to remain so until the rate of A becomes too rapid.

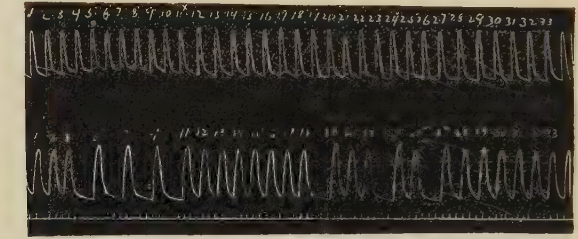

Fig. 3. Same as figure 2.

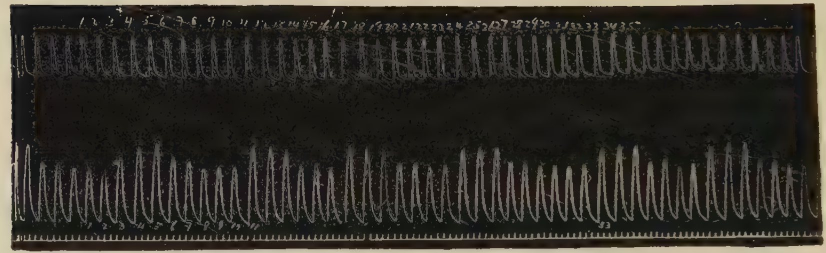

Fig. 4. A further influence of A rate on the character of V beat.

The influence of auricular pauses is still further shown in figure 4 which is a record of the same heart taken at 10.22 (table 4). The A-V interval (0.7 second added) is practically constant, showing only slight variations which, however, do not give evidence of having any influence on the V contractions. The frequency of A beat is now about

23. Every time the A pauses, to beat again with a lengthened A-A interval which gradually decreases, the following three V systoles are larger, after which the A rate becoming more rapid the strength of the impulse getting through the A-V connection is diminished and fewer fibers of the V are excited to contract and, therefore, the V systole is not so vigorous.

It is possible to obtain all grades of this type of partial block, though the most common cases are those in which every second, third or fourth V systole falls out. Many cases of the other type of partial block were also observed and tracings made showing the disappearance of the disturbance to conduction. Several points have been noted which seem of interest. In figure 5 is shown an example of the transition of a 4 : 1 to a 2 : 1 rhythm, the values of the A-V interval being 1.33 seconds before and after the transition (4.0 seconds subtracted). In figure 6 is shown the final transition to 1 : 1 (see table 3, No. 10, 3.6 to 4.0 seconds subtracted). Note that the A-V interval is increased at the time of transition. In the next column (No. 11) are given the values of a case where the A-V interval though increased at the time of transition quickly returns to its previous value. No. 20 represents another case with a marked increase in length of A-V interval as the block disappears. This at first decreases and later develops an alternation of a slightly longer and a shorter A-V interval, and a corresponding higher and lower V contraction or pulsus alternans. It is to be

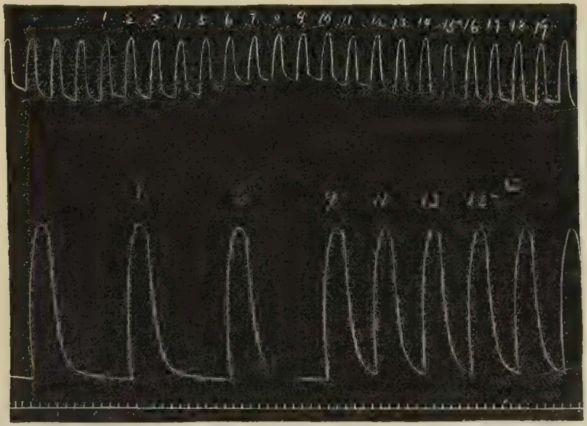

Fig. 5. Recovery of a 4 : 1 rhythm to a 2 : 1.

noted in all that at the moment of transition there is a greater or less increase in the length of the A-V interval, which may later disappear, or decrease more or less.

Sometimes the transition from a 4 : 1 rhythm takes place to a 3 : 1 (instead of to a 2 : 1) which eventually changes to a 2 : 1 rhythm. A characteristic of all cases which have been registered of a 3 : 1 rhythm changing to 2 : 1 consists in the apparent inability of the reduced (injured) A-V connection to allow the increased strength of impulse to get through once and for all. There is always a return for one or more cycles to a 3 : 1 ratio after which the 2 : 1 rhythm is permanently attained.

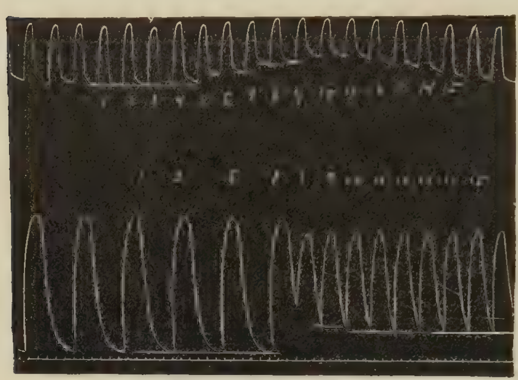

Fig. 6. Recovery of a 2 : 1 rhythm to a normal 1 : 1.

Figure 7 shows a very brief instance of this sort of thing. The V had for some time been contracting after every third A systole. Suddenly after A_s 9, the V contracted thus missing but one A beat instead of two. After that it again missed two beats and then followed regularly after every second A systole. Sometimes it takes very much longer for the 2 : 1 rhythm to be attained and the alternation between it and the 3 : 1 rhythm lasts for some time.

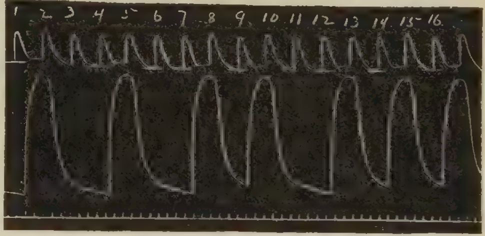

Fig. 7. Recovery of a 3 : 1 rhythm to a 2 : 1, showing the inability to make the change once and for all.

A block represented by a 2 : 1 rhythm may change, in addition to the method illustrated in figure 6, by passing through an intervening stage, characterized by the gradual increase in the length of the A-V interval (see fig. 8). At 9.37 the A and V were beating regularly in a 2 : 1 rhythm. Unfortunately the drum had to be stopped and before it was again started it was observed that not every second

V systole was being dropped, but every third, in other words the rhythm was now 3 : 2, as shown in the second part of figure 8. The values of the A-V intervals are given in table 3, under No. 18 b (0.4 second subtracted). This has become therefore a typical case of a gradual increase in length of A-V interval to a maximum after which a V systole is dropped. The records in these two figures are early stages of the block illustrated in figure 1 (see table 3, 18 a). The ratio of A and V quickly became 4 : 3. At 9.40 (fig. 1) it is 5 : 4 and soon after this the block disappeared, and coördination took its place.

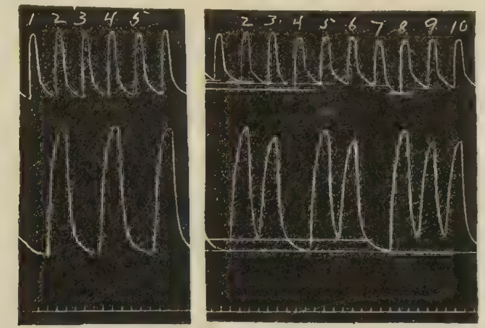

Fig. 8. A stage in the recovery of a 2 : 1 rhythm, showing intermediate conditions of 3 : 2 and 4 : 3 rhythm. Cf. Fig. 1.

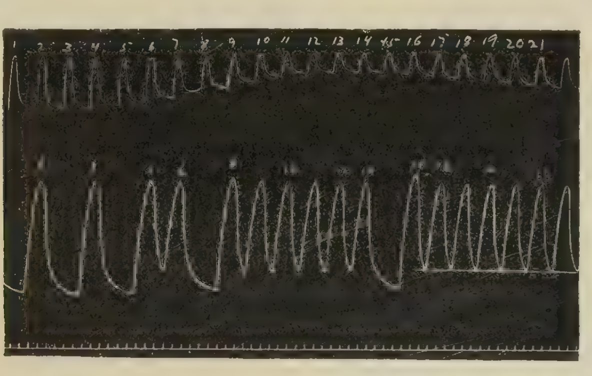

Fig. 9. Recovery of a 2 : 1 rhythm to a 1 : 1, passing through intermediate tages of gradually increasing length of A-V interval.

In No. 4 (table 3) the values of the A-V intervals of a much shorter case are shown, and in figure 9 another case is illustrated (see table 3, No. 14, 1.7 seconds subtracted). These cases are of particular interest in that they point to the view that these two types of block are closely related and that the type in which there is a gradual increase in the length of the A-V interval is intermediate between 2 : 1 block and coördinated rhythm, although a 2 : 1 block often disappears without passing through this intervening stage.

The results of these experiments are not entirely in accord with the view expressed by Gaskell to the effect that the degree of block is directly proportional to the thickness of the connecting bridge left between the A and the V, and that by gradually decreasing this, a higher degree of block is to be produced. As pointed out in the first portion of this paper, rather more emphasis is to be placed on the particular part of the connection left, since some parts of the funnel are more effective than others in conducting the contracting impulse. Partial block can of course be produced in the way in which Gaskell claims but only within limits. For if time is allowed it is nearly always seen that the remaining bridge is more functional than at first seemed to be the case, in that the degree of block decreases and finally disappears, the right and left parts of the connection having the greatest capacity for recovery.

VENTRICULAR RHYTHM

In Clemmys complete block was always to be obtained by severing the most important parts of the A-V funnel, namely, the right and left. In Malacoclemmys, as has been pointed out above, this is not the case. But complete block can very readily be obtained in this form by narrowing down the connection left after the right and left parts are cut through. This complete block may consist in the V becoming quiescent and remaining so as long as it was observed. Usually, however, the V after an interval begins to beat, at first slowly and gradually increasing in rate, but entirely independently of the A. The rate of the independent V rhythm has never been seen to approach that of the sinus rhythm, as was observed in the heart of Clemmys and figured in my earlier paper on pp. 199 ff., where in old hearts the V sometimes initiated the rhythm of the heart, and beat more rapidly than the A. (Some of these figures perhaps represent cases of very much lengthened A-V, rather than V-A). The rhythm producing power of the V, or rather of the A-V funnel, of Malacoclemmys does not seem therefore to be so highly developed as in Clemmys. But it can institute a rhythm as was shown to be the case in the lizard ((1) p.

168, fig. 33), where after the connection between A and V had been completely cut through, the V continued to beat and in the ratio of 10 contractions to 25 of the A. In one case of the development of a funnel rhythm a severe block was produced at 9.34 from which the connection gradually recovered letting through impulses so that the V gave isolated contractions which at 9.39 were at the rate of about 1 to 7 A systoles. At 10.10 and 10.20 the connection had much improved for the V was beating once to every three A beats and apparently coördinated. But a cut at 10.29 completely severing the bridge had no other effect than to slightly retard the V, thus showing the independent origin of the V rhythm. Figure 10 is a record of the heart beat after the A-V connection had been severed.

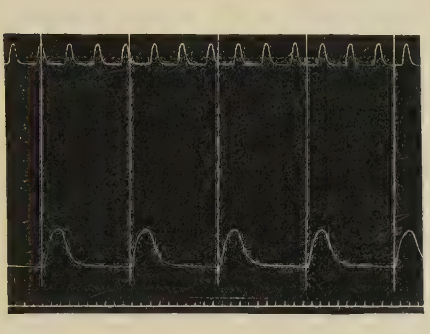

Fig. 10

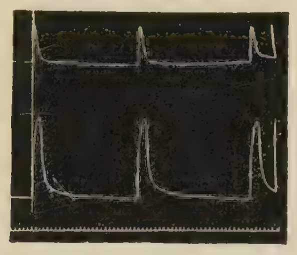

Fig. 11

Fig. 10. Independent A and V contractions after the A-V connection had been severed.

Fig. 11. An example of an A-V funnel rhythm.

To remove the possibility of this independent V rhythm being due to the Na in the Ringer solution with which the hearts were moistened a number of experiments were made in which the heart was placed in a chamber kept moist by filter paper saturated with tap-water. The results of these experiments are summarized in table 5. Several records were taken showing the rhythm originated in the A-V funnel after the sinus had been removed, and one of these is shown in figure 11. As well known, Engelmann (13) demonstrated a V-A rhythm in the frog after standstill following clamping off the sinus.

TABLE 5

Instances of the development of independent V rhythm

NO.	TEMPERATURE	FREQUENCY	TIME	OPERATION	RESULTS
52	20.5	22	2.15	First and second Stannius ligature	
		15	5.30		A is still, 1 V_s every 25–35 seconds
53	18.0	18	10.05	First and second Stannius ligature	
		17	12.20		A is still, 1 V_s every 40, 20, 35, 20, 35, 20, 35, 19, 24, 33 etc. seconds
54	18.4	21	10.30	First Stannius ligature	
		18	11.44		V and A beating about 3 per min.
			11.46		1 V-A cycle every 30, 27, 30, 35, 34, etc. seconds.
			12.45		1 V-A cycle every 30, 29, 29, 33 50, 22, 24, etc. seconds.
55	19.4	24	11.00	First Stannius ligature and V trimmed down so that only a small portion surrounding funnel remains	
		26	11.10		1 cycle every 10, 40, 27, 20, 23, 15, 27 etc. seconds.

DISCUSSION

A functional differentiation exists in the A-V funnel of Malacoclemmys geographica as in Clemmys lutaria. The right and left parts of the funnel possess in a higher degree than other parts the ability, when the one or the other is the sole connection between A and V and when reduced to a very narrow bridge, of conducting the A impulse so that A-V rhythm is coördinated. The production of severe and long lasting block when one or the other of these two parts is cut through, and the complete dissociation produced when both are cut through which was obtained with Clemmys and Lacerta have not been substantiated with this heart, and no part of the funnel can be said to be indispensable for coördinated conduction.

It will facilitate the discussion of the evidence regarding block given by the above results to briefly review the opinions of others concerning this phenomenon. Engelmann (14) explains the type of partial block, in which a V systole more or less regularly drops out after the gradually increasing A-V interval reaches a maximum, by the effect of the V systole on the length of the A-V interval. He assumes a summation of the retarding influence of the successive stimuli so that the A-V interval becomes larger and larger up to a maximum which is retained, or at which from greater fatigue a V systole is dropped. During the resting period the conductivity of the A-V connection is improved and the V contracts after the next A systole with a shorter interval or latent period, after the next A systole with a slightly longer interval and so on until a V systole is again dropped, and the process thus continued. Later (15) he concludes that the gradual increase of the latent period for excitation of the V plays a part in the falling out of V systoles which occur after the maximum value of the A-V interval has been reached, this increase in latent period pointing to a decrease in the velocity of conduction.

Muskens (16) who observed this type of block after vagal stimulation claims that it is due to blocking of the wave of excitation so that it does not reach the V, and in poorly nourished hearts, to the increased inability of the V to conduct after every V systole.

Hering (17) points out that there is no proof, excluding those cases in which the connecting fibers have been directly injured, that the dropping out of the V systoles is due to a temporary loss of conductivity on the part of the A-V connection fibers, but that the explanation is to be found in the loss of irritability of the V, in the lengthening of the refractory period (see also Straub, 18). He admits however that the gradual increase in the length of the A-V interval can indirectly cause the dropping out of a V systole by the lengthening of the refractory period of the V, but although he grants the possibility in certain cases of the failure of the A-V connection fibers to conduct causing the dropping out of a V systole, he sees nothing to induce him to accept the view. In a later publication (12) he refers the falling out of a V systole after a gradually increasing A-V interval to a retardation in A-V conduction which takes place in Tawara's node.

Von Kries (19) considers this type of block, occasional cases of which he obtained when the contractions of the V were slowed by cooling it, as due to the V systoles being shoved a little further and further apart until an auricular impulse falls into the refractory period of the preceding V systole, which results in a V systole being missed. After the rest the next V systole follows the A systole after a shorter interval and the process begins again. The impulses going from A to V must be assumed not to be momentarily concentrated but spread over a certain short period of time. Furthermore he points out the necessity of assuming in the heart muscle a refractory period for conduction after a stimulation as well as for contraction. It is interesting to note in von Kries' curve on p. 488 that the A systole corresponding to the V systole which is missed is lower than the other A systoles. Perhaps we have here an example of periodically weakened A impulses.

The other type of partial block is also thought by Engelmann (15) to be due to a retardation of conduction by which he explains rhythmic disturbances of the heart such as hemisystole and pulsus alternans. If a stimulus comes to the heart muscle at the moment when the ability to conduct has not returned to normal, then a partial contraction of the V musculature will result, and only later when all the fibers have regained their normal ability to conduct will the V as a whole take part in the systole. Differences in conductivity are not claimed to be always the explanation of such disturbances, and he admits that differences in contractility of the muscle fibers, temporarily weakened by contraction, are also important factors.

Erlanger (20) from experiments on compression of the bundle in the dog and on strips of turtle heart concludes that it is the strength of the impulse which determines whether or not it will, after having passed a partial block, serve as an efficient stimulus to the part beyond. He found it impossible to explain partial block by assuming an increase in the refractory period at the seat of injury. Furthermore, he accounts for the development of partial or complete block when the auricular rate is increased in part by the diminution in strength of the A impulses, and in part by the fact that more of the impulses fall into the ventricles before their irritability reaches the level at which the impulses of the normally beating auricles would have become efficient stimuli.

Hering (21) brings into line with his explanation of pulsus alternans this type of partial block. Pulsus alternans he considers due to the lengthening of the refractory phase of the V fibers which therefore do not respond to the stimulus. The periodic dropping out of a V systole is to be explained in the same manner, viz., by a lengthening of the refractory phase of conduction, so that the stimulus does not reach the fibers.

And finally von Kries (22), discussing block occasioned by narrowing the connecting bridge between A and V (p. 88), does not consider that merely by this procedure can conductivity be decreased, and that in order to explain the retardation in conduction it is necessary to assume a functional change in the remaining fibers of the bridge, a view tentatively stated by Gaskell (see Gaskell, p. 66). The fact that recovery from block takes place under these conditions makes this assumption highly plausible.

From this brief summary it is clear that considering the strength of the initial impulse as always the same there are two separate and distinct possible explanations of partial block. In the first the conductivity of the fibers of the A-V connection is assumed to be impaired on account of injury (actual cutting, compression or pathological conditions), so that there is a retardation of conduction or a failure to conduct on account of the lengthening of the refractory period. In the second the cause of block is sought in changes in the V, in a decrease in irritability or a lengthening of the refractory period, so that there is a failure to contract, or a failure to respond to the impulse brought to it from the A.

Both of these explanations are of course tenable, since it must be admitted that block is sometimes brought about by pathological changes in the A-V connection and sometimes by such changes in the V. Only those cases, however, in which a modification of the connection has taken place are to be considered here. It seems impossible to get away from the assumption that in such cases the resultant block is due to the injury which the fibers still present have suffered, and that some functional change takes place which retards the impulse or blocks it. Let us consider first the type of block in which the V contracts after every second, third, etc., A systole. The length of the A-V interval is increased. The strength of the impulse reaching the V is probably decreased by the cut, as Erlanger claims to be the case when the bundle is compressed, and the V therefore fails to be excited by every A impulse because the irritability of the V does not recover after every contraction rapidly enough for it to be excited. But how is the strength of the impulse decreased if not by the injury to the remaining fibers? And how is the increase in length of the A–V interval to be explained? According to Engelmann (14 and 23) we might say that the A-V interval increases because of the decreased strength of stimulus, or that the latent period of the V increases for the same reason. If the block remains stationary or even becomes greater this explanation might be sufficient. But when the degree of block becomes less or disappears, in other words when a stronger stimulus succeeds in getting through the connection, it is necessary to admit that the fibers of this connection have recovered in order to make this possible. Take a case of 4 : 1 block as it improves. When the reduction in the degree of block takes place directly to a 2 : 1, or to a 3 : 1 and finally to a 2 : 1 rhythm we note that the length of the A-V interval does not change or if it does it decreases slightly (see table 3). But when the block disappears and the A and V beat 1 : 1, it is almost invariably the case that the A-V interval is markedly increased at the time the transition is made. This increase in length of the A-V interval may quickly disappear or it may decrease to a greater or less extent but it usually remains considerably greater than when the beat was 2 : 1 (see table 3). The explanation of the improvement in A-V rhythm is that the conductivity of the connection fibers suddenly improves by recovery of the fibers from injury so that a stronger impulse gets through more often, or every time. Why the A-V interval is lengthened when the rhythm becomes 1 : 1 is possible of two explanations. A lengthening of the refractory period of the V so that it takes longer to contract or to be excited, or an increase in the refractory period of the fibers of the A-V connection. The second of these explanations is to me preferable. The impulse now getting through is presumably still weaker than it would have been if the cuts had not been made, and the fibers of the remaining bridge are still somewhat under the influence of injury. They cannot, therefore, conduct the impulse every time so rapidly, as they can every other time, the pause giving them an opportunity to recuperate their conductivity. The occurrence in many cases of a slight ventricular pulsus alternans just after the beat has become 1 : 1 is of interest in this connection. This again might be interpreted as evidence simply of a partial asystole, some of the fibers of the V not having returned to their full irritability, thus resulting in alternate V systoles being not so vigorous. But in many cases it is found that the A-V interval preceding the lower V systole is slightly shorter. This indicates that the A impulse gets through more quickly, and falls into the V at a time when some, or all of its fibers, have not regained their full irritability, and the following V systole is therefore lower. Next time the impulse, owing to fatigue of the connection fibers, takes longer to get through to the V. Its fibers are, therefore, more irritable and the following systole is larger. (No claim is made that all cases of pulsus alternans are to be explained in this way.) This condition of pulsus alternans continues until, owing to the increased improvement in the connection fibers, every impulse is able to get through at maximum speed and at maximum strength.

Now as to the other type of block in which the A-V interval gradually increases until a V systole drops out, after which the same thing is repeated. Here again the strength of impulse reaching the V is decreased, and the length of the A-V interval is increased in that the impulse travels more slowly due to the injury suffered by the fibers of the remaining connection. The general increase in length of the A-V interval from cycle to cycle is due to the gradually increased refractoriness of the connection fibers. Finally a V systole is dropped. This must be considered as due to the fact that the impulse does not get through and not that the impulse getting through is sub-minimal to the V, or that it falls into, or too near to, the lengthened refractory period of the V. After the pause the connection fibers recover their irritability and conductivity, and the first impulse gets through more quickly, the A-V interval is shorter. The V systole is of course more vigorous owing to the pause. The second impulse comes to the fibers of the bridge and finds them in a state of lowered excitability and conductivity owing to the passage of the previous impulse and this second impulse is weakened in turn and slowed, and the A-V interval is therefore still longer, and so on until the impulse is completely blocked and fails to get through.

In some cases the last V systole before a beat is missed is more vigorous than the preceding ones (see (1) fig. 49, p. 187). If the dropping out were due to the A impulse falling nearer and nearer into the lengthened refractory period of the V this could not possibly be the case; but if it is due to a slowing up and a weakening in the A-V connection, then the V would have a chance, waiting for the impulse to reach it, to recover in greater measure its irritability, so that the systole following is more vigorous. The failure of the next V systole to materialize is then plainly due to the failure of the impulse to get through to the V.

Quite often this type of block may be observed to be intermediate between a 2 : 1 rhythm and a normal rhythm, and either regressively or progressively. It has already been pointed out ((1), p. 195) that further cutting of the funnel converted a case of 3 : 2 block into a 2 : 1 block, and two cases are given above (see table 3, No. 4 and 14, and fig. 9) of the recovery of a 2 : 1 block, passing through a transitory stage of gradually increasing length of A-V interval to end with a dropping out of a V systole after which the beat becomes 1 : 1. Here again owing to recovery from injury on the part of the connection fibers the impulse able to get through the block suddenly became stronger but the connecting fibers for the time were not yet in a condition to take over every impulse without showing fatigue which they evidenced in a slowing up of the impulse and weakening it until it did not get through at all, thus causing a V systole to be missed after which a sufficient recuperation of the connection fibers took place so that the beat became normal. See also No. 18 (table 3, and figs. 8 and 1).

It has not been overlooked that a decrease in strength of stimulus alone may possibly explain both types of partial block, by the thereby relatively increased refractory state of the V, and that in the second type the gradually increased A-V interval may be explained by the gradually increasing length of refractory period of the V from cycle to cycle until an A impulse falls into it and the V cannot respond. Furthermore that the recovery from block may be simply due to an increase in the strength of the impulse getting through and the increased A-V interval to the increased length of the refractory state of the V on account of its being more often stimulated. This explanation may be the correct one, but at the present time the writer cannot rid himself of the conviction that injury to the A-V connection fibers is the ultimate cause of both of these types of block and that the failure of the V to contract is due to the failure of the impulse to reach it.

A word may be said regarding certain of my former experiments (1). In nearly all cases block was obtained by a method of gradual cutting which extended over a considerable period of time. This gave the irritability of the V time to become lowered and therefore warranted the assertion (p. 189) that the type of block characterized by a gradually increasing length of A-V interval was particularly often seen in dying hearts. Also those cases mentioned on p. 192 in which, although the A-V interval gradually increased, the V systoles did not fall out when the maximum length of A-V interval was attained, but followed the A systole after the minimal A-V interval, which gradually increased again and so on. All of these cases have been looked up and in all of them it has been found that they were truly dying, in that conditions were becoming worse instead of better, and therefore that the irritability of the V must have been decreasing. The fact noted on p. 194 that the V began to beat regularly with a length of A-V interval equal to that at which it usually fell out simply points to the recovery of the connection fibers and also to the view above expressed that the falling out of the V is not due to the A impulse coming to it when in its refractory period.

SUMMARY

1. There is a functional differentiation in the A-V funnel of Malacoclemmys as was found in Clemmys and in the lizards Lacerta viridis and agilis. When the auricles are partially separated from the ventricle, a connection on the right or left, though consisting of but a thread of tissue, in contradistinction to other parts of the funnel, can conduct the contracting impulse so that A-V coördination is preserved or restored.
2. Cutting the right or left parts of the funnel, however, does not necessarily produce block as was found in the other animals just mentioned.
3. Block produced by the method of cutting the A-V connection can be observed in all its transitional stages to complete recovery.
4. The cause of this block is believed to lie in the injury to the remaining fibers of the connection. This increases their refractoriness to receive and conduct stimuli. The strength of stimulus is decreased and its rate of conduction increased, until the impulse is completely blocked. The V fails to contract not because the impulse is too weak to excite it, but because the impulse does not reach it.

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